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Mr Desai takes on the scientists

USER departments, more relevant research, national needs . . . familiar words in Britain during the period of the Rothschild reorganisation, familiar words more recently in Australia during attempts to dismember the the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Now the Indian government of Mr Morarji Desai is going through a similar operation with its own large scientific body, the Council of Scientific and Industrial Research (CSIR). And the opposition to these moves from the Indian scientific community is extremely vocal.

CSIR is an organisation very largely conducting applied research. First established in 1942, it is most closely associated with Mr Nehru who put it on its present firm footing in 1952 and ensured, by making the prime minister its president, that the organisation had a substantial degree of autonomy, in that its director general could by-pass ministerial bureaucracy. Furthermore, Nehru made himself minister for science, and in recent years Mrs Gandhi has also held that post, so there have been the clearest signs to Indian scientists of the importance that the government attached to science and technology. In these past thirty-five years CSIR has blossomed into an organisation with forty-four laboratories including ten cooperative research associations.

The days are gone, however, when applied scientists could claim total freedom from direction. And the arrival on the scene of governments like those of Edward Heath, Malcolm Fraser and now Morarji Desai to find large expensive autonomous organisations nominally completely decoupled from departments working in the same field was bound to trigger off some very serious questioning. In cold logic many of CSIR's laboratories could as sensibly be constituent of ministries such as industry, energy, electronics and agriculture. Thus the dismemberment of CSIR with up to 28 laboratories detached to user-ministries is not without a rational basis.

Against this, however, some very serious arguments must be ranged. First, there seems to have been no consultation of scientists within CSIR before the cabinet made its decision—and thus no opportunity for the

sort of debate which might well have thrown up alternative structures. Second, CSIR laboratories are at present expected to be accessible to government and industry alike, whereas once in the control of a ministry access might become much more restricted for those not within a tight circle. This is an interesting point which in the British context would certainly be worth studying. Third, ministers with tame scientists may be tempted to use these scientists simply to bolster up their own prejudices and not for objective assessment. Fourth, India leads the world in ministerial bureaucracy and red-tape (thanks to the British), so any move that puts scientists under the control of so many paper-shufflers and rule-book-followers has to be regarded with alarm.

These are weighty objections which in the British situation were only alleviated by the placing of chief scientists of intellectual distinction right in the midst of the relevant ministries. In India, the path to new machinery may well be different but for certain it must take note of the concerns of scientists. This has not yet happened, and the government will find itself with a very dispirited bunch of scientists if it does not bring them more into the discussion.

A rather different objection comes from those who fear that the dismantling of CSIR is one stage in the process of reducing India's aspirations to technological self-reliance. CSIR has pursued many scientific projects specially aimed at cutting down the need for expensive imports, and anything that rewards the organisation for this by splitting it up is bound to be viewed with great suspicion. The Indian National Science Academy says this is an elliptical way in a statement which recognises the benefits of some foreign technology but points to the immense pools of talent available. The press have taken it up more stridently in statements such as 'CSIR has been an eyesore to the multi-nationals', and 'CSIR is the first martyr to the Janata Government's ill-concealed solicitude for the international giants'.

All this adds up to a compelling case that the government should come out and debate its proposals openly. Once CSIR is in pieces a major national asset is never likely to be put together again. □



Science versus safety: who should judge the balance?

R. N. P. Sutton, of the Department of Virology, Wittington Hospital, Manchester, offers some reflections on the possible implications of increasingly stringent health and safety requirements for scientific research in Britain

EMMA LIVRY was a young and talented dancer in the Paris of the 1860s. She chose to ignore the management in a question of non-flammable material and, in consequence, was fatally burned during a performance at the Opéra. Mlle Livry weighed art against safety and paid the price.

I doubt whether such a conscious choice could be made today. Over the years, safety regulations have proliferated, culminating, as far as Britain is concerned, in the Health and Safety at Work Act of 1974. These regulations have mandatory force and, whether *post hoc* or *propter hoc*, we now have a universal obsession with safety. Living and working in a scientific community, do we expect too much when we hope that a fair balance can be struck between a crippling devotion to safety-first on the one side and a disastrous *laissez-faire* on the other? For the moment, let us confine ourselves to microbiology, for that is the field with which I am familiar.

Do laboratory workers handle pathogenic organisms carelessly? I hardly think so. In the diagnostic field, all are well aware of the dangers of hepatitis, tuberculosis and so on, probably more so than our colleagues in other disciplines. There has been much thought about the newer hazards, Lassa fever, Marburg and rabies viruses, and the risks in handling these agents are now well recognised. Special control measures to prevent the spread of infection by these and similar agents have been set up in Britain. The Dangerous Pathogens Advisory Group (DPAG), a body broadly analogous to the Genetic Manipulation Advisory Group (GMAG), has laid down procedures for the handling of these Category A pathogens. The procedures apply to laboratories which, as a matter of deliberate policy, hold or handle or might in future hold or handle these pathogens.

In laboratories with diagnostic responsibilities there are unavoidable risks in the handling of specimens. Where large areas, maybe including major seaports or international airports, are served, the chances increase of fortuitous encounter with dangerous pathogens. Facilities are required for safe handling of specimens from patients—and, indeed, the safe handling of the patients themselves—in whom exotic infections such as Lassa fever are suspected. At this stage, the practicalities of administration and finance become paramount. The necessity of expensive preparation for a rare event does not appeal to cost-effective administrators, hard-pressed by other and urgent appeals. There is a temptation to purchase a facade of equipment and, perhaps, to designate an empty room as a high security area. Justice, but not full justice, is seen to be done. In circumstances such as these, limited by unavoidable financial constraints, clinicians, nursing staff and laboratory workers may be edged into positions which are unacceptably dangerous.

But is not all this within the domain of the Health and Safety at Work Act? Here, it is expressly laid down as a duty that the exposure of persons to risks to their health and safety is forbidden. Yes, but . . . presumably, the legislators did not intend this Act to be read as forbidding nurses to dress infected wounds, laboratory workers to test

infected specimens or doctors to attend to patients with contagious diseases.

Yet dangers exist and there would seem to be practical and financial difficulties in implementing the provision of safe conditions of work, in particular with respect to the rare, but real, hazards presented by Lassa fever and the like to the ordinary hospital and laboratory of any size. Implementation of the spirit of this law is much easier when we consider the planned investigation of known infectious agents. Here, we are often on different ground, and a fable comes to mind.

In a far away land, there was a tragic disease, well known to medical men. Rumours claimed that this disease could be transmitted to monkeys and two doctors decided to try the experiment. One told his colleagues about the exciting prospects of his investigation; these friends banded themselves into a committee and prudently forbade him to handle such a dangerous pathogen. The second doctor kept his counsel and success came his way.

I am not sure whether this fable could be held to apply to any known disease. Told twenty-five or thirty years ago, it might have applied to poliomyelitis: told today, to Creutzfeldt-Jakob disease. Times change, and it is interesting to speculate on what would have happened if the early work on poliovirus had been carried out in today's climate. Would we now have a poliovirus vaccine or would the experiments have been terminated, for the safety of the laboratory workers? Either way, patients with poliomyelitis would still have been cared for and pathological specimens subjected to routine examination without much regard for the letter of any safety legislature.

In the past, experimental risks were assessed by the scientist concerned. Accepted as being in possession of the facts, and capable of marshalling them with responsibility, his decisions were given due weight. Today this is not so and we have a society where everyone questions authority and claims the right to speak and act, often on topics beyond their competence.

In this way, bodies often effectively lay in composition can inhibit individual workers through decisions which are arrived at by processes which are essentially non-scientific (risk avoidance, responsibility spreading and undue reliance upon public opinion). Such decisions, although they may well implement legislation such as the Health and Safety Act, may equally well not be in the public interest (that is "Doing today those things that men of intelligence and goodwill would wish, five or ten years hence, had been done"—Edmund Burke).

How can we strike a fair balance between the demands of safety and those of the public interest? My concern is by no means theoretical and I know personally of recent instances where local bodies, acting in their own wisdom, have attempted, in the name of safety, to ban certain microbiological experiments. On both occasions, as it happened, the offending agents were slow viruses or possible slow viruses. Next year, perhaps, influenza virus vaccines will take over the role of bogeys for the timorous in the local safety committees—who knows?

A solution may lie in changing slightly the role of the DPAG. In the few programmes involving genetic engineering, control is rightly in the hands of the GMAG. In the many experiments involving micro-organisms, the ultimate sanction in questions of safety and the power of veto should be, not in the hands of local bodies, but in those of the DPAG. In this way both the public safety and the freedom of the investigator would be protected. □

Health and safety 3 years on

Alastair Hay traces the teething trouble of the UK Health and Safety Commission since its inception in 1974

WHEN the UK Health and Safety at Work Act (HSW Act) received the Royal Assent on 31 July 1974, a single agency—the Health and Safety Commission (HSC)—was brought into being. It was charged with securing the health, safety and welfare of people at work, and of the public out of work. The Commission has a Health and Safety Executive (HSE) to implement its decisions and coordinate the safety work previously carried out by separate agencies dealing with occupational safety and health, explosives, nuclear installations, factories and employment medical advisory services. Three other agencies were also allocated to the HSC and, in spite of considerable pressure to return them to their former status, have been retained by the Commission. They are the inspectorates for mines and quarries, farm safety, alkali and clean air.

The HSW Act states that the Commission shall consist of a chairman and "not less than six nor more than nine other members" to be appointed by the Secretary of State for Employment. At the moment the Commission has eight other members. Employers' organisations in the form of the Confederation of British Industry (CBI) appointed three, the Trades Union Congress (TUC) nominated a further three, local authority organisations two more. Although several nominations have been considered for the ninth post no single nominee has proved acceptable to both employer and employee representatives on the Commission. CBI nominees have been vetoed by the TUC representatives and vice versa. According to one member of the HSC this political infighting was only to be expected in the creation of a new organisation. It had in no way affected the work of the Commission which, he said, was "going well".

Evidence that both sides of industry are cooperating well on safety issues is provided by the safety representatives' legislation due to come into force on 1 October 1978. This provides for trades unions to establish 'safety watchdogs' in work places throughout the UK; 150,000 'watchdogs' are likely to be appointed, according to HSC estimates. When the measure was first proposed, CBI representatives opposed it, fearing that it would increase the power of trade unions; but, with no machinery other than the unions for negotiating with workers in industry, their opposition was short lived.

A further threat to the legislation was posed by expenditure cuts implemented by the government. In fact the cuts would have stopped the legislation had the CBI and TUC not put pressure on the government to secure the measure. By this time both organisations were agreed that the legislation was important. As the coal mining industry has had safety representatives at the work place for many years, the pressure is not entirely new. But its success in the coal industry is undoubtedly one reason for the HSC introducing it into others. It is also, say the HSC, the only way of ensuring that people can participate actively in decisions affecting their own safety.

Placing responsibility

The 1974 Act is quite explicit when it places responsibility for safety at work; it is the duty of every employer to ensure, "as far as is practicable", that his employees are not "exposed to risks to their health and safety". The HSE's factory inspectors have the task of ensuring that the law is enforced in the factories of manufacturing industry in the UK. In the past these inspectors had to be generalists, each one being responsible for about 500 different premises. Today there is a different strategy: inspectors concentrate on specific problem areas. A new class of specialist inspectors is being trained who will be better able to advise local safety representatives.

These inspectors are well armed too. If any employer does not comply with an 'improvement order' an inspector can issue a 'prohibition order' to stop a particular activity. In the HSC's report for 1974–76, inspectors are reported to have served 5,433 improvement notices, 1,951 immediate, and 799 deferred prohibition notices. Of the 44 appeals lodged against these notices, 31 were withdrawn, some were modified but in no case was a notice cancelled. In the HSC's view this outcome is "very satisfactory"; the Commission's inspectors are using their legal powers, but with prudence.

Prohibition notices are unlikely, however, to be served on the directors of research laboratories in the UK. According to Audrey Pittom, the Director of the Hazardous Substances Division of the HSE, "enforcement or spot checks are not necessary for research institutions". In Miss Pittom's view, scientists obviously need to exercise care in the laboratory. But she ac-



VCM worker with personal monitor

knowledges that most scientists are usually well versed in the hazards involved in their work. She says that the HSE feel that it is the large scale production processes in industry that present the real dangers. It is in these circumstances, where there is a long chain of command with no single individual responsible for the whole operation, that problems arise; this, she adds, is not the situation in the laboratory.

In laboratories where the staff are organised in trade unions, safety representatives with legal powers will be appointed by October 1978. Many well run laboratories already have safety officers so this measure is unlikely to bring about any serious disruption. It will probably be those laboratories engaged in work of a multidisciplinary nature where scientists, adequately trained in one field, can venture into another, which will come under closer scrutiny. This is an area of concern for many laboratory safety officers who argue that the technicians employed in these laboratories are often unaware of the dangers they run, either because they have not been told or simply because their employer's don't know the hazards themselves.

Genetic engineering

One of the HSC's proposals has been viewed as a serious provocation by sections of the scientific community. It concerns the Commission's all embracing definition of what should, or should not, pass for genetic engineering. The Commission feels that there must be regulations to cover this useful, but potentially dangerous research field. Its guidelines, put forward in a discussion document last year, were deliberately all-encompassing: in the words of a spokesman for the HSC it "did not want to leave anything out". The definition certainly did not do that. In fact one commentator remarked

ironically that the definition was so broad that it would even preclude consumption of a bowl of yoghurt.

But this was not the only response from scientists to these proposals. Reaction was often far more extreme. Some biologists attacked the recommendations with a fervour bordering on hysteria, principally, says the Commission, because they were not fully informed of the reasoning behind the proposals. The Commission feels that some over-reacted, others were initially slightly misguided, but that the majority discussed the recommendations rationally. Note has been taken of these discussions and the Commission is now proposing a more selective definition of genetic engineering. It has yet to be approved by the Secretary of State for Employment.

On the subject of industrial carcinogens, the HSC view is unequivocal; industry must take more effective measures to reduce the risks. Inspectors in the HSE point out that workers in specific industries are still developing cancer. More and better surveillance is necessary. The HSC uses as its guidelines the recommendations of the 59th sessions of the International Labour Conference on the prevention and control of occupational hazards from carcinogens.

As a first step to limiting the spread of carcinogens, the HSC is preparing a notification scheme for all new substances. Where the quantity manufactured in, or imported into the UK exceeds 1 tonne per year, advance information on the substance's toxicological properties must be sent to the HSC. Substances already in use before the introduction of the scheme are exempt from its provisions unless there is evidence that they present a particular hazard.

Included in the toxicological details will be information about potential carcinogenic properties. As industry will be required to do its own testing, this will have to be done according to a protocol agreed with the Commission. Animal studies are still regarded by the HSC as the most effective for determining carcinogenic potential. But as they are expensive, the Commission argues that they are not feasible as screening tests for large numbers of new substances; the short term tests now available are more practical. Two short term tests favoured by the Commission are the Ames test—for assessing mutagenic properties in bacteria—and *in vitro* cell transformation in cultured cells. It is the Commission's view that the results of these tests, as well as other factors such as chemical structure, nature of exposure and the number of workers exposed, should be considered in assessing whether addi-

tional tests are necessary. Some scientists point out that co-carcinogens are not covered by short term tests, and they add that some attention must be devoted to this problem.

The Commission is unlikely to ban substances, however, simply because they are carcinogenic. The activity of the carcinogen, its use in industry and its manufacturing process will be considered before resorting to a banning order. The Commission feels that cleaner manufacturing techniques could reduce the risk to workers from some carcinogens. Audrey Pittom cites the case of vinyl chloride monomer (VCM) as a substance, which can be "control(led) without prohibiting". VCM, an important polymer in industry but linked with the deaths from angiosarcomas of a former ICI employee and a worker at British Petroleum, is now subject to far stricter manufacturing procedures than existed in the past. Thus vinyl chloride, once even mooted as a potential anaesthetic, has escaped the prohibition notices served on some chemicals in the UK, such as β -naphthylamine and benzidine.

In the final analysis it is epidemiological evidence which is necessary for assessing the carcinogenic risk a substance carries for humans. To assist with this, the HSE, and TUC in particular, would like to see industry keep better general medical records of employees. A few specific industries do keep this information but the TUC feels that all industrial concerns should do likewise. Some argue, therefore, that it is almost certain that pressure will be exerted on the government in a year or two to introduce amending legislation to the HSW Act to secure this end.

Unlikely measure

A measure not likely to be adopted in the UK in the fight against carcinogens is the proposition put forward by doctors representing two German chemi-

cal giants—Hoechst of Frankfurt and Bayer of Leverkusen. The doctors suggested that older workers be employed in plants manufacturing dangerous chemicals on the basis that a more experienced workforce exercised more care in handling toxic substances, and that if there was a latency period of 20–30 years between first exposure and the development of cancer, these workers may be less at risk of developing cancer than their younger colleagues.

The companies made it plain that they had never adopted this practice themselves and a spokesman for Hoechst said that it might be possible to implement the decision in smaller factories, but not in one as large as his. Reaction to the proposal in Germany from the Ministry of Labour, members of parliament and trade unionists was understandably hostile; it is hardly likely to be adopted. In Britain, Mr Bill Macmillan of the Chemical Industries Association thought that the proposal was an "interesting approach" but he insisted that it was "not one we apply in Britain, nor is it one which as far as I am aware had ever been considered in this country". Spokesmen for both the HSE and one of the larger chemical unions in Britain said that the suggestion was "politically unacceptable". The real flaw in the Hoechst suggestion as far as the HSE spokesman was concerned, was that if there was a risk of someone being exposed to a carcinogen it would be impossible to restrict the substance merely to the factory confines and prevent other people in the environment being exposed.

Thus the HSC and its Executive have to look at safety measures from both a political and technical standpoint. The Commission has had to exert some political muscle to resist pressure from mining and agricultural interests to have their safety inspectorates detached



Heat exchangers at VCM plant

from the HSC and reinstated in their former guises. Attempts to have the Alkali and Clean Air Inspectorate returned to the Department of the Environment have also met with little success. But this should come as no surprise, for few governments would wish to see their own creations dismembered so early in life, and the HSC

is after all a protégé of a Labour government, albeit its second term in office.

It remains to be seen whether future British governments take the demands for dismemberment more seriously. By that time, however, most of the arguments in favour of such a measure will no longer apply, for the various in-

spectorates will be more coordinated, and existing and proposed legislation will be more in tune. As for the 1974 HSW Act, the results of that legislation can only be judged some years from now. Many are in no doubt that the Act will be shown to have achieved its objective, that is, a safer working environment in the UK. □

Protecting production or workers?

In November 1974, the British Society for Social Responsibility in Science reported in *Nature* on its work on vinyl chloride monomer. The Work Hazards Group of BSSRS sent *Nature* this update of its activities:

OVER the past three years, BSSRS has expanded its hazards programme with the aim of providing information to those directly at risk on the factory floor and to community groups directly affected by industrial hazards. We now publish *Hazards Bulletin* five times yearly which includes material on particular hazards, developments in health and safety legislation, legal cases and trade union struggles for health and safety in the workplace. Pamphlets on noise, oil, vibration and asbestos dust provide more comprehensive analyses of the effects of these hazards and how to fight them. Our hazards enquiry service now receives 50-75 enquiries per month. Local hazards groups work within trades councils, local trade union branches and community groups on health and safety issues, and we talk directly to safety representatives and shop stewards on day release safety training courses organised by concerned members of the trade union movement. This direct contact has been invaluable to our work.

While we have been developing our work at the rank and file level, worldwide concern about environmental hazards has increased. The enormity and horror of the disasters at Flixborough and Seveso, the struggle of the inhabitants of Minimata Bay to obtain compensation for the damage done by mercury poisoning, the poisoning of the state of Michigan by PBB, the militant demonstrations against fission reactors in France, West Germany and the United States, have all forced the scientific communities in industry and the universities to begin a more systematic evaluation of the hazards of old and new technologies. So far, unfortunately, this response has been grossly inadequate, reflecting a remoteness from the problem and a lack of fundamental concern for those who are directly at risk.

A conference on risk held at Imperial College, London, last May and organised by the Council for Science and Society (CSS) and the ensuing leaders and articles in *Nature* (19 May, 26 May) expressed fundamental differences of approach between our work and current academic and industrial considerations. The dominant note struck at the CSS conference and echoed in the pages of *Nature* is the need to guarantee production. Health and safety issues are secondary to the needs of maintaining and increasing production. While this approach is understandable from the point of view of those responsible for planning the economy it is unacceptable

to those directly at risk in the factories and the neighbouring communities. Significantly, trade unionists and community groups are virtually excluded from conferences such as the one organised by CSS where the issues are discussed and where policy begins to be formulated.

Aside from the inexcusable absence of those directly affected by the discussions and the decision making, there are fundamental problems when the emphasis is on guaranteeing production first and safety second. Such protection as is provided involves enclosing the worker in cheap protective clothing rather than enclosing or redesigning the production process itself. Industry sees the problem as protecting the operation and design of the process, an attitude which characterises a number of industrial approaches to health and safety.

The first approach is a reluctance to accept that a hazard exists. The asbestos industry still claims that there is no risk to the general public from asbestos (remarks of Alex A. Cross, chairman, standing committee, International Asbestos Information Conference at Asbestos Information Association Third Annual Industry-Government Conference, September 8-9, 1976). The second approach is to accept that a hazard does exist but that it is small. This leads to the notion of acceptable risk and threshold limit values (*Hazards Bulletin* 7, July 1977) in an effort to quantify the argument. In the case of asbestos, standards are set at 2 fibres per cc, which is a factor of ten greater than that demanded by the trade unions representing the majority of workers exposed to asbestos. Our evaluation of the literature leads us to give unqualified support to this very minimal trade union demand and we have urged trade unions to ban asbestos and get it replaced with the numerous safer alternatives that are commercially available (*The Asbestos Hazard*, Birmingham Hazards Group, 67 Woodstock Road, Birmingham 13).

A third argument employed when faced with an apparent contradiction between production and safety is to compare the risk with other already existing dangers. The use of fatal accident frequency rates (FAFR) frames this approach. According to FAFR statistics, which ignore occupational disease, non-fatal crippling accidents, the effects of shift work and other debilitating hazards, mining is less hazardous than driving a car. Such an argument paints a picture of the public as being irrational in opposing one hazard while seeming to accept another without protest. The *Nature* leader (19 May) supports this argument without recognising that there is no mechanism for car drivers to affect their hazard directly. The sensation

generated by Ralph Nader's book, *Unsafe at Any Speed*, shows how much interest there is in automobile safety and at the same time how difficult it is to mount a campaign for it. The crucial difference between these 'widely accepted' hazards and hazards at work is that people at work have the organisation, numbers, and power to effect change. The use of this inappropriate comparison serves to blunt the force of the basic demand for adequate health and safety precautionary measures. It is shocking to realise that for the vast majority of fatal accidents at work the hazard is recognised and the safeguards are known (*Accidents in Factories*, HMSO 1971).

The fourth and most critical argument is the claim that adequate protection is simply too costly. Mr Jack Sheppard, managing director of Turner and Newhall, one of the biggest asbestos fibre processing companies in the world, testified at the Government Advisory Committee on Asbestos that the TUC demand of 0.2 fibres per cc would close the UK asbestos industry entirely. The forced choice between jobs and the environment has been one of the most effective methods of getting people to accept unsafe living and working conditions. This can only be fought effectively by supporting the health and safety struggles in other countries and trying to prevent the export of hazardous operations to countries with less stringent requirements.

The BSSRS Work Hazards Group disagrees completely with the management approach of protecting the process. First, not only do we ask if it is safe; we ask, who is it safe for? Present industrial safety considerations are confined at best to the general public and express little concern for the workforce itself. Second, we ask, who pays for the cost of safety? If government or industry decides not to pay, this does not mean that the cost vanishes. Far from it. The individual affected pays the cost in poor health, lost wages and early death. This is a shifting of cost, not the saving of money. In the Robas Report, accidents were estimated to cost the nation 0.8% of the GNP. Third, in our practical work with trade unions we support the view that no matter how much information they may have, there is still a fundamental difference in perspective between the worker on the shop floor and the requirements of management.

It is this conflict between those who control production and those who need safety, between those who assign the risks and those who are exposed to them that must be acknowledged. There is a continuing need for scientists and technologists who are prepared to acknowledge this conflict to help make available to working people the information they need to ensure their health and safety.

EUROPE

Harmony of practice

The European Science Foundation's general assembly heard of progress last week in controlling genetic manipulation experiments. Chris Sherwell reports from Strasbourg

EUROPEAN initiatives in controlling recombinant DNA research will continue in spite of the hiatus reached in the United States, where no legislation now seems likely in the coming year. The pace set by the USA hitherto has slowed considerably in recent weeks with evidence that the dangers are less than at first expected, and the bill introduced by Senator Kennedy has now been withdrawn.

European efforts, however, though largely focused on the subject of legislation, are not directed at producing harmonisation of individual countries' guidelines. The chances of doing this are now widely regarded as negligible, more because of the work each country has put into creating guidelines suited to its own circumstances than because scientists have differed in their estimates of what are conjectural risks. Moreover, in practice the decisions of the European countries' equivalent to GMAG (Britain's Genetic Manipulation Advisory Group) have proved to be very similar across a broad range of experiments.

The aim now is therefore directed at harmonisation of practice in the individual countries. The intention is that individual representatives of national genetic manipulation advisory groups should supply lists of sanctioned experiments together with the relevant containment conditions. Anomalies would be discussed, and countries out of line would be expected at least to reconsider their position. Harmonisation would thus consist in concurring at regular meetings on decisions already taken.

The meetings, which should now take place every six months, are of the liaison committee for recombinant DNA research of the European Science Foundation (ESF). This met for the first time on 15 March this year, and again on 19-20 September. As a result of the first meeting, at which various countries' practices were surveyed, a small group of legal experts and scientific advisers met on 22 June to discuss common elements for possible controlling legislation. It considered licensing of facilities, consultation and compliance with controlling bodies, and an issue which had tormented the liaison committee meeting,

that of patents.

According to the ESF annual report agreed in Strasbourg last week, the September meeting supported the legal committee's finding that researchers' interests would be best protected if national committees requested information necessary only to assess suitable safety precautions. That the subject of legal safeguards and patents is a potential problem is revealed by the British case. Here, GMAG's dual role as both a technical advisory committee and as a watchdog for the public interest has made winning the confidence of industrialists, for whom the patents question is highly relevant, especially difficult.

Elsewhere the conflict of interest implicit in this dual role is resolved by creating a small technical advisory committee which looks at individual cases in detail and then delivers suitably edited reports to the full committee. It is a solution adopted on the European level as well: the ESF liaison committee's technical advice comes from EMBO's standing advisory committee on recombinant DNA research, which consists solely of molecular biologists.

The September liaison committee meeting also agreed on certain general points—that any effective legislation should provide for notification to some responsible body, and for licensing and inspection of facilities by that body. The idea that individual projects should receive prior approval was not unanimously endorsed, but the keeping of records and the idea of legislative review did receive support.

The liaison committee also considered technical safety issues which were the basis of a questionnaire distributed on the subject following a separate and informal meeting in June. The assistance of the EMBO committee will be sought on discrepancies between decisions of national safety committees and the revised NIH guidelines. Other areas of interest include the lowering of safety measures where the DNA to be cloned is pure, the criteria of pathogenicity for purified recombinant DNA molecules, the principles and application of biological containment, and the safety measures needed for recombinant DNA research using plant and animal viruses.

Out of all this a pan-European code may or may not emerge. For the moment, the objective is practical. And it has the useful purpose of serving the mutual interests of recombinant DNA researchers and of the European Science Foundation. □

Europe's danger

A WARNING about the future of science in Europe is contained in the annual report of the European Science Foundation (ESF), endorsed last week in Strasbourg at its third general assembly. But while the threat has not been spelled out quite so cogently before by an international scientific body, the ESF announced no comprehensive plan to help counter the danger.

The problem, a product of the mid-1970s, was summed up by Sir Brian Flowers, re-elected to the ESF presidency for another three years. If the trend of dwindling support for the important innovative research done by Europe's small scientific groups continued for too long, he told a press conference, the progress of science would be affected at the point of origin. But he confessed: "This subject was not discussed very much". He added only that it was "accepted by everybody present".

Sir Brian went on to list the assembly's achievements. Apart from moves in the field of recombinant DNA (see accompanying story), these included the establishment of a committee to conduct a feasibility study on a synchrotron radiation facility, progress of an ad hoc group on taxonomy in the fields of zoology and botany, and an elevation in the status of ESF committees on the social sciences and the humanities.

Some concern was voiced privately last week at the number of ESF committees: delegates saw a need for more tangible action. At present there is only the European Training Programme for Brain and Behaviour Research, which awards grants for travel and training, and financial support for the mathematics and physics research programme of the Institut des Hautes Etudes Scientifiques. Nor did the ESF make any statement of its position on the issue of human rights.

The ad hoc group to study the design, costs and site of a European synchrotron radiation facility will be headed by Professor Hagedoorn of the Netherlands. Its predecessor, under Professor Maier-Leibnitz, found that research using such radiation sources, which is parasitic on high energy physics facilities, had reached a stage where the lack of control undermined progress. The hope is that work will start on a European facility in 1980, to be ready by 1985. This will be in addition to such other European projects as CERN, ILL, EMBL, EISCAT, JET and the European observatories.

The decisions concerning the social sciences and the humanities add two standing committees (the former to be chaired by Jean-Jaques Salomon) to the two in existence, which embrace the science research councils (ESRC) and the medical research councils (EMRC). But the idea of helping post-doctoral students in these two areas through fellowships and workshops—which in expanded and broadened form would obviously help counter the danger to science described in the ESF report—met an obstacle over how any such scheme would be financed. It proved to be the only spark of controversy at an otherwise routine gathering.

Chris Sherwell

GERMANY

Stagnation in industry?

Werner Gries report from Bonn about statistics on research in Germany today

ACCORDING to a review presented by the federal government to the German parliament, research expenditure in the federal republic amounted to DM25,960 million in 1976, 2.3% of the gross national product. The total number of staff employed in R&D, some 303,500, has been stagnating for three years; the number of persons employed in research in the industrial sector has actually declined. Expenditure for 1977 is estimated at DM27,300 million, up 5.3% on 1976.

The government's share of total research expenditure in Germany is about 51%, industry's share about 48%. Some 60% of research expenditure is effected through government grants in the industrial sector, where the state pays an average allowance of 20% of industry's own expenditure. This support amounted to DM3,000 million in 1976, of which DM1,200 million went to defence R&D and the rest to civilian R&D. Of government expenditure on civilian R&D, 72% is handled through the federal Ministry of Research and Technology.

In 1975, the latest year for which figures are available, 186,200 persons were employed in R&D in industry. This represents a decline of 6.5% compared with the peak in 1971 of 200,000 employees, but the decrease was in the number of assistant staff and not of research workers themselves. The number of research workers rose by 8.8% over 1971-1975, and assistant staffs were considerably reduced. The decisive factor may have been the pressure of costs in the German economy. As possessors of technical knowledge, research workers were not dismissed, while the work of the assistants was increasingly done by highly trained research workers.

Industry's research expenditure is concentrated in a few firms, as evidenced by the fact that 96% of research expenditure is effected by firms which have more than a thousand employees. Government promotion of research in industry has also concentrated on those firms which have the largest research budgets. Only 6.3% of government research funds in recent years have been for the benefit of small and medium-sized firms. The promotion of research by fiscal means is at present of only minor importance in the industrial sector, because several tax concessions for research were stopped in 1975. The

proportion of funds accruing to firms via fiscal promotion of research is about 2% of the total research expenditure financed by the firms themselves.

The distribution of research expenditure among the branches of German industry bears examination. Steel construction, mechanical engineering and the motor vehicle industry used 34% of the total for all firms. These industries also received 48% of government research money for industry. The number of research personnel in these industries declined in the years 1971-73, however, from 62,100 to 54,300.

The electrical engineering, precision engineering and optical industries used 30% of research expenditure by firms. They received, on average, government allowances of 15% of their own expenditure. Research staffs in these industries have increased slightly in recent years, but electrical engineering experienced a decline from about 53,900 in 1971 to 52,700 in 1973.

The third largest and most research-intensive branch of industry in Germany is the chemical, oil and plastics sector. Some 28% of firms' research expenditure is done in this branch of industry, and it receives only 2% of its research expenditure from the state. Research staffs in the chemical industry fell from a peak of 50,400 in 1971 to 47,400 in 1973. The reduction was not in the number of research workers but in their assistant staffs.

Remaining branches of industry use only 7% of research expenditure by firms. But taken as a whole the figures indicate that stagnation in research expenditure has set in even in Germany. In the industrial sector there is a real decline in research expenditure and a reduction in personnel. It is a trend which can be observed in several other western industrial countries, except for Japan.

• The federal government will distribute DM436.3 million for projects in non-nuclear energy research in 1978, chiefly to build demonstration projects in coal refining, district heating and hot water supply from solar energy. Fundamental research will be supported in coal technology, energy conversion and the efficient use of energy.

Under a special economic programme, a number of prototype plants using new energy technology have been promoted, notably the use of heat pumps in public installations. The largest (4.65MW) gas heat pump in the world so far started working at Paderborn on 21 October 1977. □

USSR

Brezhnev's surprise

Vera Rich reports on Mr Brezhnev's jubilee speech for the sixtieth anniversary of the October revolution

MR BREZHNEV'S jubilee speech followed traditional lines for the most part but contained two surprises. The most remarkable feature of his speech was the section on reducing the danger of nuclear war. In addition to the well-reported commitment to an eventual total ban on nuclear weapon tests—to include underground as well as atmospheric, outer-space and underwater tests—he stated that the Soviet Union was prepared to agree to a moratorium on peaceful nuclear explosions (PNE).

PNE forms an important part in the Soviet plans for diverting the Siberian rivers southward to irrigate the arid steppes of Central Asia. This diversion is not entirely dependent on PNE: for explosions below 10 megatons, conventional explosives are more cost-effective, except where difficult terrain poses special logistic problems. Nevertheless, a year ago the Soviet media began to comment extensively on the use of PNE in the project, and it was envisaged as a major mark of progress. Indeed, so anxious were the Soviet authorities to use PNE in the diversion project that they were even prepared to agree to on-site American inspection. Now, however, Mr Brezhnev is apparently willing to sacrifice the whole project as the price of a test-ban treaty, and to revert to more conventional means of canal-building.

The other major surprise was the figures for this year's harvest. In spite of fervent denials by the foreign services of Moscow radio, Western experts had expected a shortfall of some 8 million tonnes. Although Mr Brezhnev made no reference to shortfall, the total yield of 194 million tonnes means that the shortfall from the target is no less than 19 million tonnes. Although the weather must be largely held responsible, it is not surprising that the future plans for agriculture stress the intensive development of agriculture.

Other present and future plans outlined in the speech include the opening up of Siberia by the Baikal-Amur railway, the new plans for developing the non-black earth zone, and "the discovery of new sources of energy and substitutes for many types of natural resources, the technical re-equipment of the economy to reduce manual and especially arduous labour to a minimum, the boosting of agriculture, the combating of disease, and the prolongation of the human life-span". □

IN BRIEF

Porton decision due

A decision on the future of the Microbiological Research Establishment at Porton is expected shortly now that the Medical Research Council has considered the complete report of its special advisory committee under Professor Sir Robert Williams and transmitted it to the Secretary of State for Education and Science. The Ministry of Defence is due to leave the establishment by 1 April next year.

The advisory committee assessed the scientific potential of Porton for civilian research. The Institute of Biology has published its written evidence in the latest *Biologist*. The institute considers that the pilot fermentation plant, enzyme laboratory and containment facilities, together with staff and ex-

pertise, should be retained, developed and exploited rather than dissipated and dispersed.

Nuffield's good turn

The Nuffield Foundation has decided to help UK scientific and medical research, by supporting projects which would normally receive research councils' money but which might go unfunded. It means a temporary change of policy for the Foundation, which was set up to support research leading to social benefit for which there is no ready source of public money. The Trustees have already allocated funds amounting to about £60,000 for research which they normally would not consider. The level of support is expected to increase next year.

In 1976, of the £1.67 million income (up almost 25%), £1.16 million was spent on support for research. Only £50,000 of that went on scientific research, however, and £70,000 on medical research. The latest annual report says one reason for the small demand on Foundation resources may have been that researchers assumed funds were low after the Foundation sold its shares in British Leyland.

NASA reorganisation

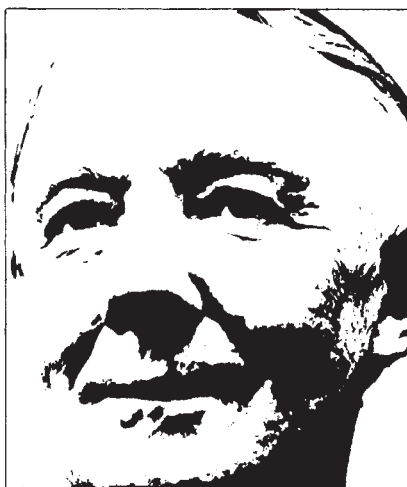
A reorganisation of NASA by its administrator, Robert Frosch, includes a revised role and title of chief scientist, who will advise on the agency's "total programme from the standpoint of scientific objectives". The change takes effect this week.

MOST people think that the only way to conserve wild plants and animals is to leave them alone. They imagine that the perfect nature reserve is an area surrounded by a man-proof fence, where natural processes can proceed without his baleful influence. This is sometimes true. The immense national parks in North America, areas like the Kruger Park in South Africa, and some reserves in the less-inhabited parts of Poland and Scandinavia require the minimum management of their vegetation, though even under these favourable conditions the larger mammals may increase beyond their optimum and require control.

The situation in countries like Britain is generally very different. We have a few large nature reserves in the highlands and islands of Scotland where nature can be left to take care of itself. Here the main management is to control man, including over-zealous naturalists who wish to collect rarities, and over-enthusiastic ecologists whose scientific sampling may destroy the vegetation. But even the most 'natural' area has probably been modified by human activity, perhaps by eliminating wolves and other predators, which allow deer to increase to numbers which ruin the vegetation unless they are severely culled.

In most areas man has reduced the tree cover and so encouraged the development of moorland vegetation. The conservationists, many of whom believe they are preserving natural conditions, oppose any move by farmers to restore the fertility destroyed by the agricultural techniques of our iron age ancestors. They produced the present beautiful—but semi-desert—landscape of, for instance, Exmoor.

Nevertheless, in many parts of Britain the native vegetation may develop if it gets the chance. The famous Broadbalk Wilderness at Rothamsted Experimental Station was a wheat field until it was enclosed and

Managing wildlife**KENNETH MELLANBY**

left alone over a hundred years ago. Soon shrubs and trees appeared, and it is now a small wood of oak, ash and other indigenous species. At Monks Wood in Huntingdonshire we started a similar experiment on a larger scale in 1960, by leaving the ten acre Stocking Close field, an area which had been arable for centuries, uncultivated. Within seven years it was covered with a scrubby growth of hawthorn and dog rose, with a scattering of oak of up to 100 saplings per acre. It was clearly on the way back to something very like the natural deciduous woodland which covered southern Britain before man

cleared it for his farms.

However, such natural regeneration may be thwarted by some of the exotic species man has introduced to Britain. When we began the Stocking Close experiment, rabbits were very scarce following the spread of myxomatosis in 1955. Today rabbits are common again, and though acorns are still scattered around the countryside by jays, pigeons and small mammals, many seedlings are destroyed. In some parts of the country the aggressive imported sycamore prevents native tree species from becoming dominant, and so management is needed to produce natural forest.

Things may be even worse in other countries, particularly in oceanic islands. In Mauritius, for instance, conservation is a nightmare. Man has been there for under 500 years, and in any numbers for 200, but he has left little of the unique indigenous flora and fauna. Though he has exterminated birds like the dodo, and removed most of the natural vegetation to grow sugar cane, it is his introductions, plants and animals, which provide the worst problems today. Where small relict areas of indigenous trees are preserved, they are swamped with the rampant growth of guava and bramble (introduced species) producing impenetrable thickets which defy control. The introduced monkeys delight in destroying the nests of the rarest birds, and mongooses in killing the most interesting mammals and reptiles. Thus conservation may provide the most difficult management problems not in developed areas where man is obviously dominant, but in an oceanic paradise where nature might be expected to control the situation.

correspondence

What is unusual?

SIR,—In Sakurai's interesting discussion of the relation between equatorial solar rotation and climatic changes (29 September, page 401), a rather unfortunate choice of terminology is used in referring to "the Earth's present unusual climatic conditions". Unusual by what standard? The best evidence of available data covering the past 1,000 years or so suggests that the period roughly from the 1920s to the 1960s was the most unusual 50-year period of the entire millennium, in climatic terms, at least for northwest Europe (H. H. Lamb, *Climate: Present Past and Future*, vol 2 (Methuen, 1977)). Studies such as that of Eddy (*Scient. Am.* **230**, 80–92, 1977) indicate that this was also an unusual period in sunspot terms, with very high peak sunspot numbers, in line with the general conclusions of Sakurai, Volland (28 September, page 400) and others.

The possibility of a return of climate towards nineteenth century conditions should not be regarded as 'unusual' climate except on the scale of a human lifetime; the unfortunate coincidence that an explosive growth of population and demand for food has happened over just that time makes it all the more important for us to develop an understanding of what really is 'normal' in climatic terms, and I would therefore urge Sakurai and other workers in this field to avoid misleading use of emotive terms such as "unusual". If the relevant sentence is rephrased to say "variation of the equatorial rotation speed may be responsible for the recent unusual climatic conditions on Earth, now returning to the normal conditions of recent centuries" the implications of the change for the activities of mankind are much more clearly apparent.

JOHN GRIBBIN

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Missions to Halley's comet

SIR,—The informative article by David Hughes (11 August, page 468) concerning the proposed fly-by and rendezvous missions to Halley's Comet was, unfortunately, less than fair to the achievements of those who have been striving to perfect various space electric propulsion techniques.

In particular, his description of ion drive and solar-sail propulsion as "... two mechanisms (that) are still at the theoretical stage ..." is certainly far from true of ion propulsion systems.

Several laboratories in the USA and in Europe have devoted many years of effort to developing ion thrusters of different kinds. The electron bombardment ionisation type, originally devised by Kaufman, has emerged as firm favourite, at least in the USA and the UK, and several versions are now ready for space qualification. These include the 10 cm diameter, 10 mN thrust T5 device developed by the Royal Aircraft Establishment, with assistance from the UKAEA Culham Laboratory and British industry, and two thrusters of 8 and 30 cm diameter, produced by Hughes Research Laboratories under a programme directed by the NASA Lewis Research Center.

The highly-efficient 30 cm thruster is particularly well-suited to cometary rendezvous missions. Its thrust of about 130 mN is appropriate to a multiple thruster, modular propulsion unit concept, with power being derived from solar arrays. This principle readily allows a wide range of throttling to match the available power as the distance of the spacecraft from the sun varies.

The application of this thruster to cometary missions has already been studied in great depth, particularly with regard to Comet Encke, and detailed designs have been produced of the necessary flight systems.

It should also be pointed out that, far from being "at the theoretical stage", two Kaufman-type ion thrusters were flown experimentally as early as 1970. They then operated successfully for 3,763 h and 2,011 h. In 1976, one was still capable of being run under its design conditions, whilst the other exhibited a single fault.

D. G. FEARN

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Engineers' salaries

SIR,—In your editorial 'Room at the top—for whom?' (22 September, page 275), you quoted, from our report to the British Association Coordinating Group, some figures concerning salaries of engineers compared to other pro-

fessional groups in manufacturing industry. The inference you draw, that salaries in production industry "... discriminate against the scientist and engineer ...", is exactly what the graphs of median age/earnings profiles appear to show, but the point we wished to bring out was that this inference is not justified.

The graphs were of median and upper quartile salaries by age for all the professional staff (22,400) in a number of leading companies. Within this total, there were 7,386 engineers compared to 607 chartered accountants and only 141 lawyers, and we were careful to give the number in each professional group. Chartered accountants are trained, and in general gain their early experience, outside industry. A limited number are recruited by industry for functions which of their nature are mainly concerned with matters at or close to Board level. By contrast, as we show elsewhere in the report, the majority of graduate engineers initially go into industry, and industry also has an overwhelming stake in those who qualify via HNC/HND and institution membership. They are employed in a wide range of jobs, from basic technical work to Board level.

Therefore, if industry succeeds in its normal job evaluation objective, to pay comparable salaries for comparable jobs, small groups of professionals selectively employed by industry will show higher median or upper quartile age/earnings profiles than a large group of professionals generally employed. It is tempting to believe that upper quartile, and in particular upper decile, graphs will show a fair comparison, but unless the samples are carefully matched in each age group this is not so.

Using exactly the same salary data, we went on to look at the number in each professional group earning salaries on or above £10,000 a year. This is one way for the companies in the survey, of answering your headline question "Room at the top—for whom?". The 749 engineers enjoying these salaries represented only 10.1% of their group, but they outnumbered the accountants, lawyers, economists and arts graduates put together.

VINCENT EDKINS

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news and views

Tubulin assembly

from L. A. Amos

THE factors controlling the assembly of tubulin into microtubules are still under intensive study, although it is now five years since Weisenberg (*Science* **177**, 1104; 1972) first found conditions in which microtubules would assemble from a brain protein extract at 37 °C and disassemble on cooling to 5 °C. Microtubule protein purified by several cycles of assembly and disassembly does not consist only of tubulin: a number of so-called microtubule-associated proteins are observed to copurify with tubulin, most of whose functions are as yet unknown.

In 1975, it was reported that tubulin purified by ion exchange chromatography would not reassemble under Weisenberg's conditions, unless at least some of the associated proteins were returned to the mixture (Weingarten, *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 1858; 1975; Murphy & Borisy *Proc. natn. Acad. Sci. U.S.A.* **72**, 2696; 1975). The accessory factors in the extract were shown to be protein and seemed to act stoichiometrically rather than catalytically in stimulating tubulin assembly. Much of the controversy since has arisen because of the considerable variation in non-tubulin components of microtubule protein purified in slightly different conditions in different laboratories. In many cases, the most prominent components appear on SDS polyacrylamide gels as a small group of bands corresponding to unusually high molecular weights (of the order of 300,000 daltons) which have been called HMW (Borisy *et al. Fedn. Proc.* **33**, 167; 1974). Other less prominent bands are observed between 55,000 and 80,000 daltons. Borisy *et al.* found HMW in stoichiometric amounts in their preparations, corresponding to one HMW molecule per 10–15 tubulin dimers, but much lower proportions of HMW to tubulin have been reported. The positions and intensities of the other non-tubulin bands seem also to vary. In preparations from Kirschner's laboratory, HMW is found only as a minor component. He therefore suggested that the assembly-stimulating

factor, which he named 'tau' was one of the lower molecular weight proteins.

Recent papers by Murphy *et al.* (*Biochemistry* **16**, 2598; 1977) and Cleveland *et al.* (*J. molec. biol.* **116**, 207; 1977) both describe the fractionation of the associated proteins into HMW and tau components. Although the two groups used quite different methods of chromatography to effect the separation, the results are in good agreement. Both found the activity present in both major fractions. Murphy *et al.* ascribe 60% of the activity to HMW and 40% to the tau fraction. Cleveland *et al.* found a third of the original activity in their tau fraction, the remainder presumably being in the HMW fraction, which in their preparations also included some very low molecular weight material.

Cleveland *et al.* have gone on to purify further the active proteins in the tau fraction and have identified a family of four polypeptides with molecular weights between 55,000 and 62,000 which they name tau I–IV. In an accompanying paper (*J. molec. Biol.* **116**, 227; 1977) these proteins are characterised in some detail and are shown to have some interesting properties, including the ability to retain their activity after being heated to 75 °C for 1 h. By comparison, the activity in the HMW fraction is much less stable. Although the tau molecules seem to be highly extended, the amino acid compositions (including relatively high proportions of glycine and proline) and circular dichroism measurements suggest a low content (12%) of α -helix. Finally, their overall charge is neutral or only slightly basic.

The latter property is rather surprising in view of the assembly facilitating activity shown by various unrelated polycationic substances, such as poly-L-lysine, DEAE dextran and RNase A (see Erickson & Voter *Proc. natn. Acad. Sci. U.S.A.* **73**, 2813; 1976), although the polymers formed in such cases are not usually normal microtubules. It has also been shown recently (Herzog & Weber *Proc. natn. Acad. Sci. U.S.A.* **74**, 1860; 1977) that pure tubulin can be induced to assemble into microtubules simply by the addi-

tion of 10 mM MgCl₂ to Weisenberg's buffer. However the critical tubulin concentration is 10 times as high (2.5 mg ml⁻¹) as in the presence of associated proteins and the resulting microtubules are considerably less stable. As Cleveland *et al.* point out, the role of the accessory proteins in assembly seems to be to shift the equilibrium for an interaction which is unfavourable under normal conditions. The manner in which this is achieved does not seem to be simply a matter of counterbalancing charges on the tubulin molecules.

The possible relationship between tau and HMW is likely to be debated for some time. When it was discovered that HMW is readily proteolysed, even during storage of the purified protein, Sloboda *et al.* (*Biochemistry* **15**, 4497; 1976) suggested that tau might consist of proteolysed fragments of HMW. From comparisons of amino acid composition and one-dimensional peptide mapping, Cleveland *et al.* conclude that tau I–IV are closely related to each other but not to the HMW material in their preparations. However, the results presented so far may not be sufficient to convince everyone; since a tau molecule derived from a specific region of HMW would correspond to only 20% of the original molecule, such a relationship would not necessarily be obvious from the present experiments.

One of the more interesting cases of abnormal assembly of tubulin is the formation, from unfractionated microtubule protein, of extended sheets, induced by low levels (0.25–1.0 mM) of zinc. This effect was first reported by Larsson *et al.* (*Expl Cell Res.* **100**, 104; 1976). The sheets offer an opportunity of observing the structure of an extended crystalline array of tubulin molecules by electron microscopy and applying image analysis techniques, including optical diffraction and computerised image reconstruction. Crepeau *et al.* (*J. molec. Biol.* **116**, 301; 1977) have carried out some preliminary studies in this direction, and have compared the structures of Zn-induced sheets and the smaller tubulin sheets formed under normal buffer conditions during microtubule assembly. In the

Zn-induced sheets, the relative arrangement of tubulin protofilaments appears to be different from that in the 'normal' sheets and in microtubules, but the protofilaments seem to be essentially the same.

The results show the structure in more detail than has been observed before, although the present resolution is still only about 20Å. Some preliminary studies on unstained sheets suggest that it may be possible eventually to achieve the sort of high resolution results obtained by Henderson and Unwin in their study by electron microscopy of the purple membrane from *Halobacterium* (*Nature* **257**, 28; 1975). □

Primate social structure and ecology

from John Krebs

GIBBONS (*Hylobates* spp.) live in pairs and defend a territory of less than 1 square kilometre; baboons (*Papio* spp.) wander over an undefended home range of 15–20 square kilometres in polygamous or promiscuous groups of about 40 individuals. These contrasts in group size, home range, mating system and so on are typical of the bewildering array of social organisations in primates, and one of the major aims of primate ethologists over the past 15 years or so has been to seek regular patterns in the differences between species.

J. H. Crook and J. S. Gartlan (*Nature* **210**, 1200; 1966) made the first serious attempt to link primate social structure with a small number of ecological variables such as food dispersion and pressure from predators. They noted, to take just one example, that ground-living primates such as baboons live in larger groups than their arboreal relatives and suggested that this could be an antipredator adaptation, terrestrial monkeys being more exposed to predators and hence benefiting more from communal defence in a large group. Crook and Gartlan clearly had the right approach, but their attempt to classify primate societies into about half a dozen ecological categories foundered because as more field evidence came to hand, too many exceptions to their rules emerged. This led to two modifications of the original approach. Some argued that Crook and Gartlan were wrong to assume that primate social organisation is always adapted to present day conditions, and that phylogenetic heri-

Nitrogen diffusion in diamond

from John Walker

THE results on conversion of diamonds from type Ib to type Ia which are reported in this issue of *Nature* by Chrenko, Tuft and Strong (page 141) have important implications for diamond physics and for synthesis of gemstones.

The Platonically ideal diamond is composed only of carbon. Real diamonds, however, contain impurities, and paradoxically, the large, regularly shaped and apparently perfect gemstones are usually the most impure. (This effect is well-known to crystal-growers—impurities affect the shape of many types of crystal.) In diamond the most important impurity is nitrogen. A diamond is classified as type I if it is appreciably impure, and as type II if it is relatively nitrogen-free. The nitrogen may be present as isolated individual atoms (type Ib), giving the diamond a yellow colour, a paramagnetic resonance signal and a characteristic infrared absorption; or it may be in aggregates, especially pairs (type Ia), resulting in no paramagnetism, no visible colour and a different infrared spectrum. This type-classification is qualitative, and diamonds of mixed type are often found, but it is useful nonetheless.

The vast majority of natural diamonds are type Ia, but synthetic crystals are usually type Ib. Nobody knows why for sure; a plausible hypothesis is that some natural diamonds at least, started life as type Ib, and were subsequently transformed to the Ia type.

Diamond is the metastable form of carbon at normal temperature and pressure. It is stable, and so can be synthesised, at higher temperature and pressure. The hypothesis is that, in some conditions, although nitrogen can be incorporated into the growing crystal, it is immobile; subsequent higher temperatures or lower pres-

ures allow it to become mobile and diffuse through the carbon lattice until it can aggregate with other nitrogen atoms. Chrenko *et al.* report that after annealing at 2,000 K and 60,000 atmospheres for 30 min the type Ib diamonds had become appreciably paler yellow in colour, due to reduction of their characteristic visible absorption, and that their paramagnetism was weaker. The infrared spectrum had changed from typical type Ib to mixed Ia and Ib, and the N3 ultraviolet absorption system (thought to be an aggregate of three nitrogen atoms) could be detected. The total nitrogen concentration remained constant—a useful consistency check.

What is the significance of these results? First, they tell us more about how nitrogen behaves in diamond; and they may help us to understand other defects, especially the 'platelets'—laminar defects on {100} planes which have intrigued and puzzled diamond physicists for 40 years. Second, they indicate a method of turning yellow synthetic diamonds into 'water-white' ones, thus enhancing their value as gemstones. Third, they tell us more about the synthesis process, which may lead to the commercial synthesis of large gem-quality diamonds. (At present, although tons of synthetic diamonds are produced annually, they are suitable only for industrial applications—it is actually cheaper to dig the gemstones out of the ground than it is to synthesise them.) Finally, the results may have implications for the conditions prevailing in the Earth's mantle that led to the natural synthesis of diamonds. As Sir Charles Frank put it, 'Nakaya has written a poem in which he says, "A snowflake is a letter to us from the sky." A diamond is a letter to us from the depths, and a letter more worth reading because we can visit the sky.'

tage plays a strong role (Struhsaker *Folia Primat.* **11**, 80; 1969) while others proposed that Crook and Gartlan were in principle right, but that the differences in ecological pressures had to be analysed on a much finer scale (Clutton-Brock *Nature* **250**, 539; 1974), and further that the same ecological pressure may lead to different solutions.

In an important new review, T. H. Clutton-Brock and P. H. Harvey (*J. Zool. Lond.* **183**, 1; 1977) have greatly extended and updated Crook

and Gartlan's original approach. They have classified all the primate species for which they could find data into nocturnal or diurnal, arboreal or terrestrial, and insectivorous, frugivorous or folivorous. They then did a quantitative statistical analysis to look for consistent differences between these ecological categories in features such as group size, home range, sex ratio and body weight. The main trends to emerge from their analyses were that nocturnal primates which are all arboreal and mainly eat either

fruit or insects, are small, live in small groups and have small home ranges; both diurnal and nocturnal fruit eaters have large bodies, and live in larger groups and home ranges than leaf eaters; and finally, diurnal terrestrial primates live in larger groups and ranges and have more marked sexual dimorphism than do diurnal arboreal species.

How are these general trends related to ecological needs such as food gathering and avoiding predators? Clutton-Brock and Harvey suggest that the nocturnal species have to have a small body because they feed by crawling on to small branches, and they rely on crypsis to escape from predators. At the other extreme, conspicuous terrestrial monkeys living in open habitats have evolved large body size partly as an antipredator device, and partly to allow them enough mobility to search over a wide area for scattered food. Although Clutton-Brock and Harvey agree with Crook and Gartlan's idea that large groups in terrestrial primates may be an antipredator adaptation, they also point out that there are large differences in group size within both the arboreal and terrestrial categories. They propose that larger groups are always advantageous in diurnal primates, perhaps as an antipredator device, but that competition for food sets an upper limit to group size, so that species feeding on large clumps of food can afford to live in larger groups than those eating more evenly scattered food. A comparison of two types of Colobus monkey, one of which eats fruit, flowers and shoots (highly clumped food), and the other which eats leaves of all ages (less clumped) supports the idea: the former live in larger groups.

Home range size is obviously closely related to group size and is probably determined both by the minimum area needed to support the group, and by the density and dispersion of food. Species feeding on evenly dispersed, fairly dense food supplies have small, often defended home ranges, while those eating widely scattered clumps of food tend to have large undefended ranges, for obvious economic reasons.

So far, the differences in social structure seem to be well explained by straightforward ecological pressures, but it is not so easy to account for the variations in mating system and adult sex ratio. Monogamy is very rare in primates, and it must presumably have evolved only when a male significantly increases his output of young by helping the female (for example, monogamous marmosets have twins and the male helps to carry them), or because the male cannot secure more than one

mate. (This may seem an unduly chauvinistic view, but the sad fact is that female mammals as a whole have little choice about who does the parental care.) The second of these possibilities may account for monogamy in species such as the gibbon in which the even distribution of food favours territorial defence, and males may be unable to defend enough food to support more than one wife. The majority of primates breed in harem or multimale groups, and the ecological factors favouring one system over the other (and the general bias of adult sex ratio towards females) are not at all clear. It is, however, apparent that strong sexual dimorphism (σ : ϕ weight ratio) is related to the degree of bias in the sex ratio and to polygamous or promiscuous mating. This suggests that sexual dimorphism in primates has evolved when males compete for the chance to mate.

Clutton-Brock's and Harvey's review emphasises the fact that it is possible to account for many of the differences in primate social structure by pressures associated with detailed dispersion of food and vulnerability to predators. It also reveals that the various dimensions of social organisation, such as group size, sex ratio and so on, may be influenced by different ecological factors and should therefore be analysed separately. One of the next steps will be to see to what extent variations within a species in different habitats can be used to test the ideas generated by interspecies comparisons.

Biochemical oscillators

from N. MacDonald

OSCILLATORY phenomena in organisms, with periods of seconds or minutes, are known in great variety. The most obvious example is the heart pacemaker oscillation in the membrane potential of the sino-atrial node. Oscillatory membrane potentials also occur in neurones, in cells secreting hormones, and in smooth muscle. The interpretation of such phenomena in terms of underlying oscillatory biochemical reactions presents a challenge to both experimentalists and mathematicians. Identification of the steps in a feedback loop is not sufficient, unless the likelihood of instability of the steady state is established. Formidable

difficulties arise from the nonlinear dynamics of enzyme-catalysed reactions, from the length of the chains of reactions in feedback loops, and from the linking of these loops in complex networks.

In seeking to reduce networks of reaction equations to manageable proportions one relies heavily on the fact that the time scales for biochemical reactions vary widely. Retaining for detailed study those reactions with relaxation times rather smaller than the period of the oscillation, the other reactions may be subsumed into parameters. Particular interest then attaches to the values of these parameters at which qualitative changes occur in the nature of the solutions of the equations retained. In mathematical terms, this is the field of bifurcation theory. Alternatively one may retain more equations, but classify them into fast and slow sets. Then it is necessary to explore situations in which smooth changes in the slow dynamics lead to qualitative changes in the nature of the fast dynamics. This is the field of catastrophe theory, as discussed in this context in the recent review by Heinrich, Rapoport and Rapoport (*Prog. Biophys. molec. Biol.* **32**, 1; 1977).

Both the review by Heinrich *et al.* and that by Goldbeter and Nicolis (*Prog. theor. Biol.* **4**, 66; 1976) are largely concerned with detailed models of oscillations in the glycolytic pathway. Here direct evidence of periodicity in the concentrations of chemical species is available, and a great deal of work has been done in constructing and testing models. The models examined in these reviews stress the activation and inhibition of the enzyme phosphofructokinase by one or more of the adenosine phosphates AMP, ADP and ATP.

Much progress has been made recently in the mathematical analysis of the single loop, end-product inhibited reaction sequence advocated by Goodwin (*Adv. in Enzyme Regulation* **3**, 425; 1965) as a paradigm for a biochemical oscillator. One highlight in this is the theorem of Hastings, Tyson and Webster (*J. Differential Equations* **25**, 39; 1977) which is applicable to a wide class of such loops, and allows nonlinearity in all the equations. This theorem states that instability of the steady state solution is a sufficient condition for the existence of at least one periodic solution, although unfortunately this solution need not be stable. Another highlight is the systematic application of approximate methods by Rapp and Mees (*Math. Biosciences* **25**, 165; 1975; *J. math. Biol.* **3**, 203; 1976 and in the press) to estimate periods and amplitudes of these solutions.

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Factors tending to promote instability of the equilibrium point, and the onset of the attendant oscillations, are of three kinds. Strong nonlinearities, such as can be expected with allosteric enzymes, help. So do the presence of a large number of successive reactions in a loop, and the similarity of the time scales of all these reactions. These two factors go together, since widely different time scales allow some of the reactions effectively to be ignored. There is still a wide gap between the appealing idea of the end-product inhibited oscillating loop, now put on a firm mathematical basis, and the presentation of a fully worked out and experimentally testable scheme, possessing these factors, for some specific oscillator. End-product inhibition is rife in cell chemistry, but this can be understood solely in terms of advantageous properties of steady states. In a well-defined sense (Savageau *Biochemical Systems Analysis*, Addison Wesley, 1976) these states have minimal, which implies optimal, sensitivity to parameter change.

A bold attempt has recently been made by Rapp and Berridge (*J. theor. Biol.* **66**, 497; 1977) to implicate, in a wide range of oscillations, end-product inhibited loops involving the binding of calcium ions. They propose two different styles of loop, involving calcium in conjunction with cyclic AMP. In the first an increase of cyclic AMP causes an increase in cytoplasmic calcium, either by promoting calcium entry through the cell membrane, or by causing a shift in cell calcium from a bound state to the cytoplasm. Cytoplasmic calcium on the other hand is taken to inhibit the synthesis of cyclic AMP, acting through the inhibition of the allosteric enzyme adenyl cyclase. The alternative loop reverses the activating and inhibiting roles of cyclic AMP and calcium.

Sustained oscillations have been observed in the transepithelial potential of the salivary gland of the blowfly *Calliphora*. Rapp and Berridge cite evidence that here cyclic AMP can release calcium from some intracellular store, and that this store may be in mitochondria. They also cite evidence that calcium can inhibit cyclic AMP synthesis in this gland, and that this may be by inhibition of adenyl cyclase, for which there is evidence in certain cells of a variety of organisms. On the other hand, they argue that the second kind of loop may be responsible for the periodic contractions of smooth muscle. They also examine cardiac pacemaker oscillations, membrane potential oscillations in cells producing insulin, and signalling in the slime mould *Dictyostelium*.

In all these contexts their evidence is

cumulative, seeking to implicate calcium as significant in contexts in which oscillations occur rather than proposing detailed dynamics. It is a somewhat daunting thought that each context may require a similar amount of effort to that devoted to glycolytic oscillations over the years. But the work of Rapp and Berridge should focus critical attention on a particular set of possible common mechanisms for a variety of periodic phenomena. □

Acceleration of auroral particles

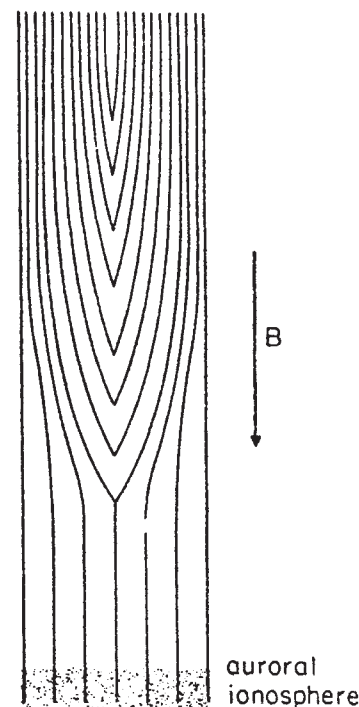
from Alan Johnstone

AURORAL electrons are accelerated into the upper atmosphere by electric fields parallel to the magnetic field lines with the total potential difference sometimes exceeding 10 kV. The fact that the acceleration is caused by a quasi-static electric field, and the magnitude of the potential difference can be deduced from measurements of the angular and energy distributions of the precipitating electrons. The electric field increases the electrons' velocity parallel to the magnetic field leaving the perpendicular component unchanged. This creates an angular distribution which peaks in the downward direction parallel to the magnetic field. This contrasts with the angular distribution of the source population of trapped electrons which usually has a minimum along the field. All electrons are given the same increase in energy (e times the potential drop) so that any observed at lower energies must have been either backscattered from the atmosphere and then reflected from the potential, or scattered out of the beam by wave-particle interactions. As there are fewer of these electrons the energy spectrum develops a peak close to the energy corresponding to the potential differences. Sounding rockets and satellites have found that such electron distributions are common above auroral arcs. The acceleration is found to be greater in the centre of the arc than on the northern and southern borders implying that the equipotential surfaces in a meridional slice above the arc are V-shaped as in Fig. 1.

The accelerated electrons have been detected at altitudes up to 1,500 km and ions accelerated in the opposite

direction have been detected by Geostationary satellites near the equator, but the altitude where the particles were accelerated could not be located until the S3-3 satellite was put into an elliptical polar orbit with apogee at 8,050 km and perigee at 260 km in 1976. In a year of operation it has traversed the auroral zone at all altitudes between these extremes. Convincing evidence from this satellite that particles are usually accelerated somewhere in the range between 2,000 km and 7,000 km was one of the highlights of the recent IAGA meeting in Seattle. P. Mizera and J. Fennell (Aerospace Corp.) showed examples of upward-moving, magnetic-field-aligned ion beams accelerated below the satellite by several kiloelectron volts detected simultaneously with downward-moving electron beams accelerated by comparable amounts above the satellite (Fig. 2). This is a clear indication that the satellite is in the middle of the acceleration region. The ion beams include both O^+ and H^+ ions (Shelley *et al. Geophys. Res. Lett.* **3**, 654; 1977), confirming that they originate in the low-altitude ionosphere. The probability of observing an accelerated ion beam in a traversal of the auroral oval is as high as 95% at altitudes above 7,000 km and less than 5% below 2,000 km. What the data do not tell us is how the electric field is

Fig. 1 A schematic diagram of the equipotential contours above an auroral arc. The innermost contours are negative so that electrons are accelerated downward parallel to the magnetic field B into the auroral ionosphere (Swift *et al. J. geophys. Res.* **81**, 3931; 1976).



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distributed in this height range. It could all be confined within a narrow layer, as some theories require, or it could be distributed evenly over the entire length. If the layers are thin then at least two must exist on the same field line in order to give the simultaneous observation of upward accelerated ions and downward accelerated electrons. F. Mozer (Berkeley) presented measurements of the ambient electric field from the same satellite (see also *Phys. Rev. Lett.* **38**, 292; 1977). The magnitude of the electric field, in structures which he termed 'paired electrostatic shocks', was more than 0.5 V m^{-1} perpendicular to the magnetic field with a smaller parallel component. This is far greater than the field strengths found below 1,000 km. The structure in the paired electrostatic shock is just the same as would be found traversing the V-shaped potential of Fig. 1 and the potential difference between the centre and the edges can reach several kilovolts if it is treated as a purely spatial variation. The accelerated ion beams and paired electrostatic shocks are found in the same region but the detailed correlation is not close. The ions spread over a region more than five times the thickness of a typical electric field structure. If they were accelerated upward by a V-shaped potential distribution beneath observed electrostatic shocks they would only be found in the centre of the structure itself. The detailed comparison of the electric field and particle observations is still at an early stage. The results of analysis now underway will be interesting and significant.

These data do not discriminate between the various proposed physical mechanisms which could be maintaining the kilovolt potential differences. Until the observational evidence for parallel electric fields became overwhelming it was thought that the plasma conductivity was too great to allow potentials of more than a few volts to develop along a magnetic field line. Strong electric currents flow upwards along magnetic field lines above auroral arcs (carried by a downward flux of electrons) which may exceed the current density the plasma can support with low resistance. Three mechanisms have been proposed to explain the formation of high potentials with strong field-aligned currents. The electrostatic shock layer, which has been shown to exist as a shock-like solution to the Poisson/Vlasov equations for strong currents, has a thickness of the order of an ion gyroradius (D. Swift, Alaska). This is related to the double-layer, also current-controlled, but not much thicker than a Debye length. Anomalous resistivity

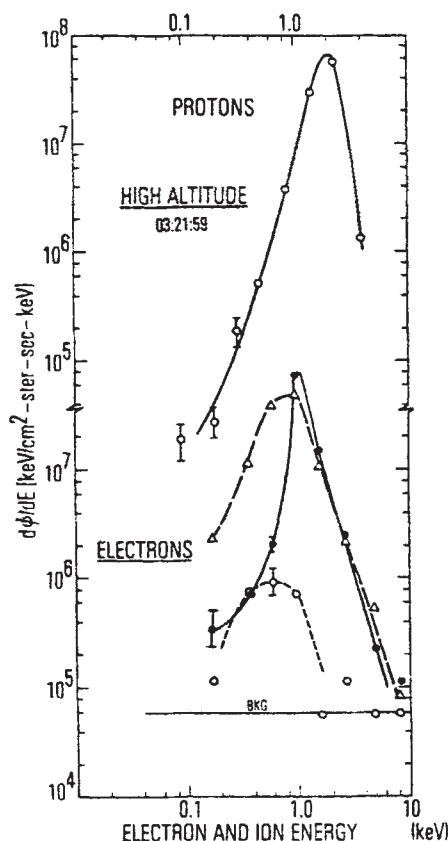


Fig. 2 Differential energy flux spectra for protons and electrons at 7,300 km altitude over the northern auroral zone. $\circ$, Upcoming particles; $\bullet$, downgoing; $\triangle$, mirroring. The mirroring electrons below the peak may be trapped between the converging magnetic field on one side and the electric potential on the other. (P. Mizera and J. Fennell, Aerospace Corporation).

is created when the current flow becomes unstable to the generation of electrostatic ion cyclotron waves. The resultant electrostatic turbulence hinders the electron flow. Finally, high voltages can be required to drive a current in a hot plasma down the converging field lines against the magnetic mirror force.

Evidence for all three mechanisms can be found in the S3-3 data but none of it is conclusive. C.-G. Falthammar (RIT, Stockholm) suggested that more than one of them may be operating. For example, the plasma turbulence associated with anomalous resistivity may help the formation of a shock layer.

If it is becoming clearer how the parallel electric fields might be maintained, it is still not clear how the contours above the V-shaped layer in Fig. 1 close, and where the energy source which drives the field-aligned currents is to be found. It was noticeable at the IAGA meeting that most scientists discussing auroral acceleration mechanisms ignored those regions of the magnetosphere above the acceleration region and that those con-

cerned with auroral substorm-related events in the far plasma sheet and geomagnetic tail ignored the influence of the ionospheric ends of the magnetic lines of force. The time has come to try to fit the two ends together. $\square$

Microanalysis in biology and medicine

from Patrick Echlin

The Third International Conference on Microprobe Analysis in Biology and Medicine was held in Münster on 5-8 September, 1977 under the auspices of the German Electron Microscope Society and the Royal Microscopical Society. The papers will be published by Hertzl-Verlag, Stuttgart, later this year.

The previous meetings in 1973 and 1975 showed that a wide range of microbeam analytical techniques could be used in biological investigations. The meeting in Münster demonstrated that the methods are at last providing physiologically significant data from a wide range of plant and animal tissues, which are in close agreement with similar data obtained using ion selective electrodes, flame photometry and chemical analysis.

In many instances microbeam techniques are taking analysis beyond the limits of more conventional methods, as was clearly shown in papers on the application of electron probe X-ray microanalysis to cells and tissues. T. Hall (University of Cambridge), claiming that the spatial resolution was now as low as 20 nm and the performance better than 10^{-18} g of an element, showed how X-ray microanalysis could be used for quantitative analysis of diffusible ions in frozen-hydrated sections of fluid-transporting epithelia. A. Somlyo (University of Pennsylvania) was able to show, using freeze-dried sections of smooth muscle, that the high cellular chloride in this tissue was not due to compartmentalisation in organelles and that the sarcoplasmic reticulum is a major storage site of calcium in both smooth and striated muscle.

G. Kirk (University of Chicago) demonstrated how the electron probe could be used in the analysis of single cells, by showing how the membrane transformations which distinguish red blood cells with a naturally high level of potassium from those with a

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naturally low level of potassium occur early in the differentiation of the erythroblastic stem cells. A. Lauchli (Technical High School, Hannover) using frozen-hydrated bulk root tissue, has shown that sodium is reabsorbed from the vessels in the proximal regions of the roots of certain plants.

Discussions on specimen preparation for microanalysis led to the conclusion that, with the possible exception of tightly bound elements, conventional wet chemical procedures were to be avoided and that low temperature techniques involving frozen-hydrated or frozen-dried sections, bulk samples or microdroplets of cell fluids were to be preferred. The only compromise might be to use freeze-substituted material or to encapsulate specimens in high molecular weight cryoprotectants before freezing. P. Echlin *et al.* (University of Cambridge) demonstrated that these polymers, which do not enter the cell, limit the size of intracellular ice crystals to nanometre dimensions, and do not seem to cause any significant loss of elements from cells and tissue, although some elemental redistribution may occur within the cell. The extent of elemental redistribution generally remains a problem, but there was agreement that valid elemental concentrations can be measured between different cell compartments, although it is probably premature to try and measure gradients and variations in concentration within a given compartment.

Several papers dealt with the quantification of analytical results. J. Russ (Prairie View, Illinois) described a new and relatively simple computer program for processing and displaying elemental spectra obtained from thin biological samples, and T. Appleton (University of Cambridge) discussed ways in which analytical information obtained from frozen-dried sections could be related to the fresh hydrated state. B. König (Batelle Institute, Frankfurt) considered the optimal conditions for high spatial resolution in the analysis of thin films and R. Bauer (University of Munich) gave details of a program for thin sections of biological tissues which separates elemental peaks and background radiation by means of artificially generated spectra. Using this technique together with internal albumin standards, R. Rick (University of Munich) found evidence for a syncytial sodium transport compartment in all epithelial layers of frozen-dried sections of frog skin epithelium.

There have been significant advances in the development of the laser microprobe mass analyser. R. Wechsung (Leybold-Heraeus, Cologne) gave details of a commercially available in-

strument with a spatial resolution of 0.3 μm , detection limits of 10^{-20} g within the 0.1–100 p.p.m. range and the capability of analysing both organic and inorganic material. F. Hillenkamp (University of Frankfurt) and R. Kaufmann (University of Düsseldorf) demonstrated how the instrument could be used to analyse trace elements and physiological cations and anions or for fingerprinting organic constituents.

Unfortunately, this high level of sophistication is still missing from the techniques of cathodoluminescence, and in the nuclear and proton probes. These techniques, together with ion probe analysis, still need further development particularly with respect to improving their spatial resolution and their biomedical applications. These are clearly the analytical techniques of the future and for once the biologists are ready to use them, having firmly established valid preparative methods based on low temperature techniques. □

Economy and chemistry of phosphorus

from P. B. Tinker

A symposium, entitled The Economy and Chemistry of Phosphorus was held at the Ciba Foundation on 12–15 September, 1977, and chaired by Professor R. J. P. Williams. The Proceedings will be published by the Ciba Foundation.

PHOSPHORUS occupies a special place amongst the elements which are essential to life, because of the variety and complexity of the processes in which it takes part. The wide-ranging symposium was therefore of the broadest interest.

The first and most basic topic was supply, since there have been suggestions that phosphorus is a mineral resource which may be near exhaustion. Several speakers stressed the underlying problem of the explosion of population and economic expectations. The present facts are not in dispute: current production is some 15 million tons each year, rising on a long term trend of 6–7% per year, from minimum demonstrated reserves of some 6,000 million tons, which suggests exhaustion in 50 years. However, real reserves must be far larger, and J. W. Brinck (International Resources Consultants,

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Alkmaar), used a modelling approach to infer that there could be anything from 40,000 to 500,000 million tons in all. Even this largest value would be exhausted in 120 years if the trend continues. As with all futurology, the numbers are so inexact that one can do little except show that rising trends cannot be sustained for ever. The real question is the mechanism by which these rising trends are stopped. As Brinck remarked 'Doomsday is always 70 years ahead,' and the best estimate seems to be that we have enough phosphorus in mineable reserves to take us beyond the limit of useful prophesy, especially since recent price increases have sharply reduced consumption. Possible technical developments in mining technology, the use of sea-floor phosphatic nodules, greater efficiency in the use of fertilisers and phosphorus recycling make prediction especially difficult, and it was suggested that our problems are more likely to be the familiar ones of ill-directed investment or the exercise of monopoly power. The analogy with oil springs easily to mind, but it is not really a good one: oil has several substitutes, although its withdrawal would be catastrophic at once, whereas phosphorus could be cut off without serious immediate harm, although it has no substitute at all.

By far the greatest part of mined phosphate is used for fertilizers, and G. H. McClellan (International Fertilizer Development Center), described the technology of their production, stressing how this affected the possibility of using low-grade ores.

The phosphorus cycle had a good run, with E. J. Griffith (Monsanto) discussing it on a global scale, J. C. Bowman (University of Reading) giving very detailed data for Britain, and A. F. Harrison (Natural Environment Research Council) dealing with discrete ecosystems. Natural ecosystems of forest and grassland have most of their phosphorus stored in the soil, with very small outputs and inputs, whereas the cycle in agriculture is much more 'open', with large inputs (as fertilizer) and outputs (as harvested crop). However, it is incomplete, in the sense that the inputs exceed outputs by a factor of between 2 and 10, due to adsorption of phosphate in the soil. It became quite clear during the meeting that this is the central problem in the world phosphorus economy, which consists largely of digging out phosphorus at one place and storing in the soil at another. The magnitude, value and use of this phosphorus store is not wholly understood, since the physical chemistry of phosphate in soil is extremely complex, but it is crucially important.

R. J. P. Williams (University of

Oxford) introduced the consideration of the detailed role of phosphorus in biology, noting the vital functions it performs in the transfer and storage of information (as nucleic acid) and energy (as ATP) and in compartmentation (as phospholipids). B. C. Loughman (University of Oxford), followed with a detailed description of the uptake and metabolism of phosphorus by plants, the most notable point being the enormous ability of organisms to concentrate phosphate from extremely dilute solutions. This is important in the utilisation of 'soil store' phosphate, and crops can take this up, although it may not be able to support maximum yield. There is some possibility of breeding crops which are more efficient at absorbing low-solubility soil phosphate, and microbiological systems such as mycorrhizas are often able to assist phosphate uptake.

The importance of this uptake ability of organisms was illustrated by the very interesting and occasionally contentious section dealing with eutrophication of surface waters by phosphorus wastes—largely from detergents in sewage. The discussion centred on the Great Lakes, and G. R. Alexander (US Environmental Protection Agency) discussed the problem in general and political terms. There is agreement that phosphorus is the limiting element in all five Great Lakes, and removal of phosphorus from sewage inflows has proved unreliable. He saw little chance of stopping any of the other sources of supply, such as topsoil erosion, and a ban on detergent phosphates in sensitive areas would be the easiest method of improving the position. Members of the relevant industry naturally did not entirely agree. A much more relaxed view was taken by R. W. Collingwood (Water Research Centre, Medmenham Laboratory), who felt there was no justification for the high cost of removing phosphate from sewage in Britain. Our disposal problems are very much easier than those in America, and he emphasised that each case should be treated on its merits, rather than imposing blanket bans. The subject is extremely complicated, including political judgement of what 'eutrophication' is acceptable, and the great difficulty of predicting exactly what may happen in the sometimes complex ecosystems of lakes. On the whole, the real damage to the community resulting from either algal growth in rivers or from the withdrawal of polyphosphates in detergents seemed rather small in relation to the often passionate interest aroused by it. The very useful point was made that 'eutrophication' is really a state of higher biological productivity, which may be positively desirable, and that we should not

simply assume that the less phosphate the better, without thought.

The chemistry of phosphorus underlies all the other topics, though it was difficult to deal with it in depth in such a broad-based meeting. A. J. Kirby (University of Cambridge) discussed the fundamental facts of the transfer of the phosphate group, which is the essence of its biological importance. This deals with the P-O bond, and contrasted with the paper of T. D. Inch (Microbiological Research Establishment, Porton Down), who discussed the interesting compounds with P-C bonds. The phosphonates are important in the nerve gases and pesticides based on these, and they do occur in both plants and animals, though in very small amounts. Some of their substitution reactions are interesting in giving a retention of steric orientation.

R. J. P. Williams finally wound up the Conference, with a look back at the problems of unwanted phosphorus storage, and the desirable and undesirable growth resulting from its presence. The meeting produced no radically new ideas, but it formed an excellent synthesis of the state of knowledge at present. The wide spectrum of disciplines and interests made the discussion always interesting, sometimes fascinating, and showed up very clearly the different mental approaches, for example between the pure chemists and the 'field' scientists. □

Photosynthesis at Reading

from J. Barber and B. Halliwell

The Fourth International Congress on Photosynthesis was held at Reading on 4–9 September, 1977. The Congress dealt with all aspects of research into photosynthesis under two broad categories; the light reactions, and carbon metabolism (including the physiological and applied aspects of the subject).

A SERIES of symposia brought together biophysical and biochemical work aimed at understanding how energy capture, transfer and trapping occurs in photosynthetic organisms. This area is highly active at the moment due chiefly to the development of new techniques and to the increasing use of

sub-chloroplast particles and purified reaction centres from photosynthetic bacteria. For example, G. Searle (Imperial College, London) reported how time-resolved transfer sequences between different pigments can now be studied in photosynthetic tissue using picosecond fluorimetry. P. Thornber (University of California, Los Angeles) emphasised that picosecond absorption studies are also providing valuable information on primary charge separation in bacterial reaction centres, and in particular referred to recent work with *Rhodospseudomonas viridis*. As yet, purified reaction centres from O_2 -evolving organisms have still not been isolated and direct comparisons between these systems and the photosynthetic bacterial system can only be speculative.

Although the 'Z-scheme' for electron flow in O_2 -evolving systems seems to be the best working model, there is still confusion about the role of cytochrome b_{559} , cytochrome f and cytochrome b_6 . The function of these components must be understood before it can confidently be said that a full description of electron transport in photosynthesis is known. Moreover there may be other electron transfer components which have not been identified. The existence of a new component was suggested at the congress by R. Malkin (University of California, Berkeley). He reported that a 'Rieske' iron-sulphur centre exists in chloroplasts and from several lines of evidence suggested that it functions as an electron carrier between the plastoquinone pool and plastocyanin. Plastocyanin now seems to be fully accepted as a major electron carrier in chloroplasts and at the meeting the results of high resolution X-ray analysis of its structure was reported for the first time by H. C. Freeman (University of Sydney). Knowledge of the mechanism by which molecular oxygen is generated from water by light energy is progressing steadily. T. Wydrzynski (University of Illinois) presented some new results obtained with colleagues based on NMR studies. Their results suggest that the 'S-state enzyme' of the 'Kok-clock-model' contains four manganese atoms and is advanced by Mn^{2+} to Mn^{3+} changes. Whether their model is correct or not is open, but efforts to gain a chemical understanding of the reactions involved are welcome and moreover their model fits with studies of flash-induced H^+ release presented by others at the meeting.

Photophosphorylation

The symposium concentrating on photophosphorylation clearly demonstrated that in its broad terms, Mitchell's chemiosmotic scheme is ac-

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cepted by most workers. A few papers were presented which disagreed with some of its finer points and the exact values of the H/e or P/e ratios are still not known. Over the years H. Witt (Berlin) and his colleagues have presented strong experimental support for Mitchell's concepts and again at this meeting, Glaber from Witt's laboratory reported experiments in which ATP synthesis had been induced by creating artificial electrical gradients across chloroplast membranes. A requirement of the chemiosmotic scheme is that there is a membrane-located ATP synthetase or coupling factor able to do work when translocating protons down their electrochemical potential gradient. The coupling factor consists of several subunits and N. Nelson (Haifa, Israel) reported some unique experiments. He seems to have isolated the 'H⁺-ionophore' component of the complex and has tested its ability to increase H⁺ conductance by incorporating it into artificial membrane systems.

Carbon metabolism

The sessions on carbon metabolism dealt largely with the regulation of CO₂ fixation in C₃ plants and with photorespiration. No great advances in our knowledge of C₄ metabolism were reported, but it is now generally accepted that the Calvin cycle operates only in bundle-sheath cells. CO₂ is fixed in the mesophyll, transferred to the bundle-sheath as malate or aspartate and released again to be refixed by the Calvin cycle.

Regulation of the Calvin cycle is achieved by several mechanisms, one of which is a light-dependent increase in the activity of certain enzymes. Illumination generates dithiol groups within the chloroplast, which then activate the enzymes. L. Anderson (University of Illinois, Chicago) and B. Buchanan (University of California, Berkeley) gave evidence that both membrane-bound dithiol groups and the dithiol protein thioredoxin seem to

be involved. A tentative mechanism may be proposed (Fig. 1).

As. H. Heldt (University of Munich) reported, when chloroplasts are illuminated, the Mg²⁺ concentration of the stroma increases by 1–4 mM and its pH from 7.0 to 8.0 as H⁺ enters the thylakoids and Mg²⁺ moves out. These changes regulate several enzymes of the Calvin cycle, especially fructose and sedoheptulose diphosphatases. Both enzymes work best at high pH and Mg²⁺ concentration. Ribulose diphosphate carboxylase requires preincubation with Mg²⁺ and CO₂ to obtain maximum activity *in vitro*: it is not clear if the enzyme is always fully activated *in vivo*. According to D. Walker (University of Sheffield) the kinetics of the activated enzyme can now account for observed rates of CO₂ fixation. J. Preiss (University of California, Davis) suggested that starch synthesis is regulated at the level of ADP-glucose pyrophosphorylase, which is activated at high phosphoglycerate/inorganic phosphate ratios.

Illuminated chloroplasts generate H₂O₂ but contain no catalase activity. Ascorbate peroxidase together with glutathione reductase may remove H₂O₂ *in vivo*, an argument put forward by D. Groden (Bayreuth) and by B. Halliwell (King's College, London).

Photorespiration is caused by the formation of glycollic acid and its subsequent oxidative decarboxylation. G. Lorimer (Munich) reported experiments with ¹⁸O₂ which show that most, if not all, of the glycolate is produced by the hydrolysis of phosphoglycolate generated by the oxygenase activity of ribulose diphosphate carboxylase. U. Heber (Dusseldorf) also carried out other experiments which supported this conclusion. The glycolate so produced is oxidised to glyoxylate and transaminated to glycine in peroxisomes. The glycine is converted into CO₂, NH₃ and serine in mitochondria. As A. Moore (King's College, London) explained glycine oxidation by leaf mito-



A hundred years ago

THE chief signal officer of the U.S. army has been urging that physical observations of the sun be made, as of sun-spots, faculae, protuberances &c., in reference to their supposed influences upon terrestrial meteorology, and has offered to publish the results monthly, or such of them as may be considered desirable by the observer, in the *Monthly Weather Review*. The United States Naval Observatory at Washington has already accepted this proposition, and it is considered very desirable that some other observatories in the east and at least one on the western coast, cooperate in this undertaking.

From *Nature* 17, 8 November, 39; 1877.

chondria has a P/O ratio of 3; the NH₃ formed is probably a substrate for glutamine synthetase. Several groups of workers have shown that the amount of carbon flowing through glycine and serine in illuminated leaves is more than sufficient for glycine decarboxylation to account for observed rates of photorespiration (D. Canvin, Queen's University, Ontario; C. Whittingham, Rothamsted). However, Canvin also reported that the ¹⁴C-labelling kinetics of glycine and serine are extremely complicated and inconsistent with a single origin for these amino acids, so it cannot be clearly stated how much photorespiratory CO₂ arises by glycine decarboxylation.

Refixation of CO₂ released by photorespiration consumes considerable energy within the leaf. G. Krause (Dusseldorf) thought that photorespiration may help to use up 'excess' light energy and protect the chloroplast from damage. As. E. Elstner (Munich) explained, the electron acceptor complex of photosystem I can reduce O₂ to O₂⁻, the toxic free-radical superoxide (Elstner, Asada). In the absence of CO₂ NADPH/NADP⁺ ratios in the chloroplast will be high and so electrons should be shunted more rapidly to O₂. Photorespiration, by making CO₂ continuously available for refixation, might help to decrease O₂⁻ formation to a level that can be dealt with by the chloroplast's protective mechanisms. □

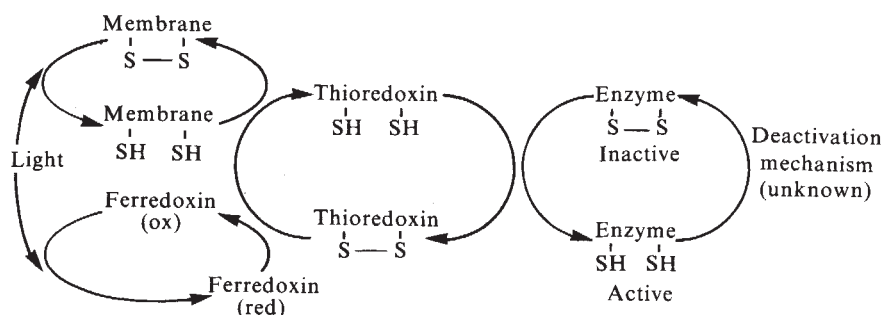


Fig. 1 Tentative mechanism for activation of Calvin cycle enzymes by dithiol groups generated by illumination of chloroplasts.

Book Review Supplement

THIS year marks the centennial of Edison's phonograph; next year is the centennial of the Edison Electric Light Company (which General Electric claim as their birthdate); and 1979 will mark the hundredth anniversary of Edison's incandescent bulb. It would be possible to go on to suggest plausible Edison celebrations for each year well into the twenty-first century, thus giving strength to the claim that here we have the most prolific inventor of all time. Furthermore, many of his discoveries were fundamental contributions to devices which now dominate our lives, thus giving firm support to Mr Clark's subtitle.

The perspective of a hundred years should make it possible for the author, as promised on the jacket of the book, in writing about Edison "to present him clearly against the background of his times and to assess fairly what his achievements really were." There are occasions in the book where a gesture is made in this direction—as on p14 where Edison's characteristics are equated with those of his age, or in the few instances of reference to the financial manipulations of his robber-baron contemporaries. But none of this is pursued. There are no fresh insights into the relationships between Edison and his family, or Edison and his workmen, or Edison and people like Jay Gould. There is the suggestion that after his first wife died his re-marriage to someone quite different significantly affected his attitude towards his work (p146). But even this is not amplified, and references to his work habits in later years seem to indicate a single-minded dedication virtually unchanged from the earlier period.

What we have, therefore, is a pleasantly readable biography, conventional in form, which offers no special commentary beyond what is available in other works. To me, it is not as satisfactory as the biography by Matthew Josephson, published almost twenty years ago (*Edison*, McGraw-Hill, 1959). This is partly due to the fact that Josephson allowed himself twice as many pages as Clark, partly due to the research he did on the Edison papers, and partly due to his familiarity with that period of American economic history. But Clark's biography shares with Josephson's a major failing in not describing in any significant detail the inventions that lay

at the heart of Edison's life. It is therefore difficult for the reader to assess the nature of the challenges facing Edison, or the ingenuity with which he solved them. There are a number of other more specific failings as well, and it seems worthwhile to mention a few of them here.

There is, for instance, the marvellous story of how Edison received a telegram from London in December, 1878, urging him to develop a telephone receiver that would circumvent the

Ushering in the electronic age

Bernard Finn

Edison, The Man Who Made the Future. By Ronald W. Clark. Pp.256. (Putnam: New York; Macdonald and Jane's: London, 1977.) \$12.95; £6.95.

Bell patent. (Unmentioned is the fact that in Britain Bell's patent protection extended only to the particular devices invented, whereas in America he was allowed credit for the principle of voice communication by a fluctuating electric current.) In a *tour de force* that was so typical for him Edison did what was asked. He shipped off a working model within three months, thus allowing his supporters in England to arrive at a profitable understanding with the opposition. Clark tells this story, and tells it well. After noting, however, that this was a time when intensive efforts were being expended on the incandescent lamp, he tries to make a point about Edison's methods of operation, stating that he "acted promptly and ruthlessly. He withdrew all his men from work on the electric light and put them on telephony." (p59). But this was surely not the case. The new receiver (which depended on the varying amount of friction that was produced when a varying current passed between a rotating drum and a stylus pressed on it) was directly related to an earlier telegraphic instrument he had invented; and although Edison undoubtedly expended a good deal of

additional thought and experimentation, and called for some assistance, there is no reason to believe he would turn the entire laboratory over to the project. Furthermore, during January, in the middle of this three-month period, Edison produced his first high resistance lamp.

Clark's account of the development of Edison's carbon-resistance telephone transmitter is not so much in error as it is misleading. Although noting (p58) that the patent application was made in 1877 and not granted until 1892, he dismisses the delay by stating merely that "it is symptomatic of the controversial situation." This was a very important invention (as Clark recognises), and it would seem reasonable to include some discussion of the controversy and of the parallel work of Emile Berliner, Francis Blake, and others.

Nicola Tesla, that most peculiar electrical genius, is given credit (p159) for developing the transformer and motor that made alternating current transmission practical. Although there is a considerable amount of truth to the statement, it suffers greatly from oversimplification, and a few qualifying words would seem in order. This is especially relevant because for the period in question—the late 1880s—the important work (on the transformer) was carried out by William Stanley for Westinghouse, with some debts owed to earlier activities in Hungary and Britain. On the same page, Clark relates that in 1912 Edison and Tesla were offered jointly the Nobel Prize in Physics, that Tesla spurned it because of his ill feelings towards Edison, and that it therefore went to someone else. This is a story that has several versions; the one which is Clark's source even has the date wrong. Whether the Nobel committee contemplated giving the prize to them is unknown, but in November 1915 the *New York Times* printed an account that this was the case. Others followed, and there were of course interviews with the two principals. No known statements by Tesla indicated any reluctance to accept the award; when asked why Edison might be honoured, he responded that Edison was worthy of a dozen Nobel Prizes.

The whole matter of the AC-DC controversy is given some prominence by Clark—and rightfully so—as marking a change in Edison's way of



Photo: Edison National Historic Site, Orange, New Jersey

Edison with two of the lamps produced to illustrate the Edison effect, later to become the basis of the electronics industry

thinking. This was during the period, already mentioned, when he remarried; an analysis of how various pressures then came to bear on Edison, and how he reacted to them, would make extremely interesting reading. Unfortunately, there is no real attempt to do this. Even the account of the succession of events is marred by errors of omission and commission. For instance, unmentioned by Clark, there were some very good reasons supporting Edison's side of the argument: storage batteries could be used in conjunction with direct current generators to even out the load cycle, and no motors had (yet) been invented to work on alternating current. The eventual capitulation—a merger with Thomson-Houston—is presented (p179) as if Edison were completely unaware of what was transpiring. Although it is true that Edison was against the merger and was largely kept in the dark, he had been aware for some time of the state of negotiations. His strong reaction, cited by Clark, was to the revelation of the final details, which merged everything together and stripped his name from the resulting firm title of General Electric.

There is no reason to believe (p65) that weakness in theoretical training

caused our hero to miss an opportunity to exploit the Edison effect. This was his discovery in 1883 that negatively charged particles were given off by his lamp filaments, and that they could be attracted to a positively charged extra electrode. He carried out a number of experiments and then applied for a patent where the effect was used in a sensitive current detector—the only use he could see for it. The experiments were known to physicists and electricians throughout the world, and none had a better suggestion. John Ambrose Fleming experimented with Edison-effect bulbs over the next few years before also abandoning them. It was two decades after the discovery (1904) before Fleming applied the Edison effect to act as a moderately effective detector of radio waves, and another decade before such a device (with a third electrode, added by Lee de Forest in 1906) was successfully used in an amplifier, thus ushering in the electronic age.

There are numerous other examples of this same type, and if one were to draw a moral from them I suppose it would be that scientific and technical biographies are very difficult to write. Further evidence for such a statement is the fact that very few have been written. If anyone should know this, it should be Mr Clark, who has specialised to a degree in this sort of writing. In his recent biography of Einstein he minimised the weakness of his own scientific background by doing a considerable amount of legwork, seeking out unused written material and interviewing people, to provide the makings of a dimension to the person he was describing that had not been available before. This could have been done with Edison. If Clark had used his strengths as a biographer to probe Edison's character and his dealings with other people, then the results might have been of great interest.

The fact of the matter is that the corpus of Edison material—most of it preserved at the historic site in West Orange, New Jersey—is enormous. The task of dealing with it intelligently in its present form, in spite of the amount of organising effort that has already gone into it, is far beyond the capacities of any one person. A fitting centennial tribute to Edison would be the inauguration of a project to develop these papers in a more useable fashion and to make them more widely available. There is hope that this will happen. If it does, the next biographer will be able to reveal to us a great deal more about the man who made the future. □

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Don Brothwell

Origins: What New Discoveries Reveal About the Emergence of Our Species and Its Possible Future. By Richard E. Leakey and Roger Lewin. Pp. 264. (Macdonald and Jane's: London; Dutton: New York, 1977.) £8.95; \$17.95.

THERE is perhaps some wisdom in the idea that before books are written, the authors should discuss the contents with potential reviewers. In the case of a detailed monograph or lavishly illustrated coffee-table book, one knows pretty well where one stands in terms of how to review it. In the first case one evaluates it particularly in relation to the limited number of specialist academics or students who will consider it; at the other extreme, the acid test is whether it will hold its own against Scotch, conversation or the television. But the hybrid is a problem, and that is really what *Origins* claims to be. The publishers tell us that it is "handsomely illustrated and fascinating to read" as well as being "filled with controversial ideas and fresh insights". Oh dear, a difficult one, pitched at the general reader but putting out new ideas which are presumably for professional eyes as well. The only answer is of course to try and play the same game. Is it pleasant reading for any of us interested in the evolution of our species (and most of us pretend to be, even though the state of 'advanced' societies suggests that few really are)? Secondly, does it really contribute something new to this well-trodden field, beyond a few new pictures and a few extra basic facts (easy enough to produce in human palaeontology)?

But to begin at the beginning, the sequence of chapters follows a pattern which is fairly standard now in books concerned with the evolution of man. We see primate evolution scaled against the few thousand million years of emergent life generally, a fact which should really put us firmly in our place and give us a strong perspective for viewing the biosocial absurdities of our own species; but it never seems to. The history of evolutionary controversy and theory is scanned briefly, reminding one that bias is all too easy and that there is a need for a detailed study of the history of palaeoanthropology, with

not only Darwin, Huxley, Owen and Dart, but also Elliot Smith, Ruffer and many others who were not only concerned with evolutionary theory but were also working on such questions as neurological change and ancient disease—exotic perhaps to some but just as relevant to the proper appreciation of the biology of past hominid populations.

Viewing the primates as a whole over their seventy million years or so of evolution is also an exercise of use to the hominid palaeontologist, setting his own distinctiveness and rates of change against that of other species within the Order. There are clearly explosions of adaptive success; first the prosimians, then monkeys, followed by a rather modest hominoid (ape-plus-human) differentiation, and finally the population explosion of modern man himself. In the interests of easy reading, considerable opportunities are missed for showing the questionable nature of much of the work and findings in this field, and the great research challenge which remains. But this is always the problem in popularising science, and although perhaps a lot of the fun of research is in the uncertainties, challenges and points of disagreement with colleagues, perhaps this does not lead to good reading at a general level? I confess that I think it does, but admittedly detailed and truthful revelations about some controversial issues would have to be very skillfully and carefully written indeed in order to avoid multiple libel actions.

Although much of the book seems to highlight Africa as a focal area of palaeoanthropology, the chapter on hominid beginnings rightly points out that most apparent ramapithecine material, a possible early hominid of about ten million years ago, is found outside Africa. This seems to be a rather neglected yet very significant fact, and if this group is indeed part of a variable stock from which the later hominids were derived, there is no reason why, in the following three or four million years, this group could not have given rise to advanced hominids in Europe or Asia, rather than Africa alone.

The next chapter, "The Cradle of Mankind", is concerned with a further morphological phase seen in human evolution—the australopithecine and *Homo habilis* groups of Africa. But how can one be sure, in this early stage of field-working world Plio-Pleistocene deposits, that many sediments out of Africa will not eventually produce these or related hominid varieties? And even if relevant fossils do not appear soon in other continents even though many more do in Africa, what does this

mean? From the evidence of skeletal material excavated in Britain, one would have to conclude that the Scots and Welsh hardly existed and only southern Britain was generally populated over the past few thousand years—a fact really correlated with soil acidity rather than historical demographic truth.

As expected, the fossils grouped under the name of *Homo habilis*, and especially the 1470 skull from Lake Turkana, are discussed in some detail. The fact that a number of deposits have now produced evidence of this "primitive *Homo* of nearly four million years old" is certainly interesting but whether "Just a few years ago no-one in their right mind would have believed this possible" seems debatable. I for one considered it possible and am not noticeably of unsound mind.

If one views human evolutionary studies from the turn of the century, it can be seen how the timescale has been greatly extended. In this respect at least, we tend to be rather conservative, although there seems to be an easier acceptance of much larger dates now, and indeed there is perhaps even a growing feeling that we must give as much time as possible for the mosaic of changes we know to have taken place in the limbs and pelvis, skull and brain. For, whereas Sir Arthur Keith, writing fifty years ago, could consider morphological evolution as if it were a sequence of easy growth changes, we must now see it as a complex product of multiple gene pool changes linked with environmental demands and social development.

Early tools are rightly given space in various parts of the book, and the comments on the early evidence is of course especially interesting. The great problem is to know what some of these roughly made objects really mean in terms of cultural development in the community. We can watch nest construction in birds or object manipulation in chimpanzees until we are blue in the face and still be quite unable to usefully interpret the significance of a remnant stone technology in another species where the brain is known only from an endocast. What is particularly tantalising is that one suspects that tools such as the poorly manufactured quartz objects from the Omo River Valley are telling us more than we can yet cope with in terms of early hominid perception and language development, the education of the early hominid child into the practicalities of hunter-gathering, and even the emergence of more advanced forms of group co-operation.

With the discovery of the fairly complete *Homo erectus* cranium from

East Turkana, doubts as to the considerable antiquity of this species are rather cleared away. (Claims for an earlier date from Java have been viewed with some uncertainty.) Whether Turkana Man was "very similar" to Peking Man is rather debatable, but that does not affect the importance of this find, and I would have liked to read more comments on this and related African specimens. For that matter, the authors could have usefully drawn in more of the *Homo erectus* and later Middle Pleistocene specimens which have been discovered in the past decade or so; and indeed I felt that the book sadly underplayed some discoveries in non-African areas of the world. I confess to being reluctant to accept their scheme for the emergence of *Homo erectus* in East Africa and then derive European and South-East Asian representatives from this stock. Perhaps this is only the result of a suspicious mind refusing to believe that the early hominids of between two and five million years ago were so restricted to Africa.

More than one chapter is concerned with this level of evolution and in the

one on "Intelligence, Language and the Human Mind" the authors give an opinion that "the vocal apparatus of *Homo erectus* would have enabled him to speak in a slow and rather 'clumsy' fashion". This brief but understandable comment is perhaps the essence of popular writing and perhaps does no harm, even though it is very misleading as regards the certainty of this fact and the lack of good evidence to substantiate it.

After considering the more recent phases of human evolution, including such aspects as population increase, agriculture, aggression and rituals, the authors are still able to conclude optimistically. And perhaps it is all to the good if palaeoanthropologists do cultivate a good bedside manner, cheering us on instead of placing us in the shadow of the dinosaurs. Unfortunately, I suspect that our mission may eventually be that of Job's comforter.

Leakey and Lewin have produced a nice book for the general reader, not one for the student or professional. □

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Fanatical system-builder

A. J. Cain

The Spirit of System: Lamarck and Evolutionary Biology. By Richard W. Burkhardt, Jr. Pp. 285. (Harvard University: Cambridge, Massachusetts and London, 1977.) £11.55.

It is a great relief to have at last a good book on Lamarck in English, neither partisan through patriotism, nor distorted by dislike of Darwin. Burkhardt gives us an excellent introduction to Lamarck, his contemporaries and predecessors, and to the scientific bodies and institutions in revolutionary Paris. Anyone working on Lamarck is necessarily limited by the almost total lack of biographical and personal material, but Burkhardt has gone carefully through the published writings and the manuscripts of Lamarck at the Musée d'Histoire naturelle, and those of Cuvier there and at the Institut. He has read widely in the literature of the period, and his sketches of the ideas and opinions of Daubenton, Faujas de Saint-Fond, Buffon, de Jussieu, Bruguière, and Lacepede, for example, are essential for understanding the variety of opinions and the wonderful richness of the biological, physical and chemical work in Paris in Lamarck's lifetime.

On Lamarck himself Burkhardt gives a useful sketch of his life and career, and the best analysis both of his evolutionary ideas and the way in which he altered his opinions, or at any rate some of them. The title of the book refers to that passion for system building (*esprit de système*) which had already been recognised as a major obstacle to scientific progress—Lamarck would not have liked its application to him at all, but it is justly used. He was a fanatical system-builder, far too hasty, far too impatient to test the validity of the 'facts' he built on, far too tenacious of dubiously correct ideas, and incredibly opposed to the best physical science of his day. He was, in short, a first-class

crank. One is reminded of Alfred Russel Wallace on anti-vaccination, life on Mars, spiritualism, land nationalisation, and the flat-earth people. Cranks are usually disastrously wrong, but when they are right, because of their fanaticism they are righter than most others—in some respects Wallace's *Darwinism*, which rather surprised Darwin, was better than the *Origin of Species*; and he was of course a great biogeographer. Lamarck also has plenty of claim to biological credit, for his taxonomic work; but his system of thought on the nature of life and on evolution reposed on such hopeless physical, chemical and geological foundations, and was presented with such disregard for scientific sobriety, that it is no wonder he was rejected by his contemporaries. One cannot but have sympathy with him for his unfortunate life, his blindness, his miserable last years; nevertheless, he was a crank. Burkhardt approaches him with too much deference to say anything of the sort, but he does indicate at the end that Lamarck cannot be considered a man ahead of his time.

Lamarck began his scientific work as a botanist, adept at constructing keys and fully aware of their artificial nature, believing in the immutability of species, and convinced that within the plant and the animal kingdoms separately there is a serial order which is the natural order. He knew well that both plants and animals vary considerably, often in relation to different climatic or cultural circumstances. He knew, as did everyone, that there was a balance in nature, such that all species continue, acting as checks on each other by predation; none, he believed, were lost. Two of these ideas, somewhat battered, he clung to to the end of his life—that within animals or plants there was a single natural serial order, or perhaps two; and that except for some large animals perhaps extinguished by man and the hordes of infusorians which could be easily destroyed by variations in external conditions, no extinction had taken place. It was this last conviction that, as Burkhardt shows very clearly, drove him to the idea of mutability of species—if it could not be that large numbers of species had become extinct, then they must have transformed into other species.

Right from the beginning, Lamarck had been thinking about the nature of plants, their constitution and physiology. He was especially impressed with the influence of climate, soils, and other environmental factors on them, and he started to think out a general theory of living and non-living things. It would be fascinating to look at his Jesuit schooling, which might

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in part explain some of his ideas in physics and chemistry. He clung all his life to a thoroughly idiosyncratic four-element theory (of earth, air, fire and water) combined with those subtle and invisible fluids, magnetism, electricity, and so on, which were the common property of almost all scientists in the eighteenth century. At first he thought of the origin of the world, and the nature of motion and of life as wholly mysterious, but before he finished he could explain the powers of life entirely in terms of his own physics and chemistry. He also believed that matter without the action of life had no tendency to form compounds. Complex matter left to itself would continue to disintegrate, and he produced a degenerative series beginning with excretions, secretions and corpses, ending with the purest of all solid matter, rock crystal. Burkhart considers the well-known antagonism between Cuvier and Lamarck to be a consequence perhaps of the former's religious views but also because he had a philosophy of stability antagonistic to Lamarck's ideas of change. It is as likely that Cuvier was a better scientist than Lamarck and that his ideas were better founded in the practical science of the period.

Lamarck's observations in geology had convinced him that the Earth had undergone very long slow gradual changes; he was an ultra-uniformitarian and therefore could think of no mechanism for large-scale extinction. He was also a good conchologist, and Burkhart brings out well the importance of this subject for problems of extinction at the time—Faujas de Saint-Fond, struggling with it, was so eager for Lamarck's conchological system that he worked from the proofs of it. On the one hand, many species seemed to be extinct but to be related fairly closely to present ones; on the other, forms thought to be extinct occasionally turned up alive (a trigoniid claim in Australian waters, for example); and vast areas of the world were still unexplored. It was a difficult question, more so for Lamarck because large numbers of intermediate forms, which should have existed on his theory, had not come to light. Why he should have clung so tightly to non-extinction is not explained. I suspect it was because, as an ardent Deist he could not contemplate serious imperfection in the world. His over-riding faith, that everything happens by law, surely springs from this attitude and from the gigantic success of Newton. Probably, his belief that the time was ripe for a synthesis of all biology from the facts then available came from an unfortunate hint of Newton's that also influenced Erasmus Darwin deeply.

Once convinced that transmutation of species must have occurred, Lamarck immediately found a dual mechanism for it, a purely physico-chemical system, whereby life, once started in simple specks of matter, would through the circulation of fluids cause expansion, then channels for the fluids, then further complexities of organisation. This, by itself, would produce a uniform series of living things beginning daily with spontaneously arising animalcules, and ending after an incredible period of time with Man, the most perfect of all. But each animal, or plant, has to live in particular ways, and their circumstances would act to modify them for their roles. This would explain neatly why there is, ac-

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cording to Lamarck but not Cuvier, an overall progression in the great groups of animals or plants but much difficulty in making serial arrangements of species or genera within them. This mechanism of adaptation was again a commonplace of the time, the inheritance of acquired characters. Lamarck said explicitly that he prided himself not on this mechanism but on seeing its enormous implications, which no-one else had done.

Lamarck had no reputation in France until it was necessary to find a national rejoinder to Darwin. Much of what was then written was uncritical in the extreme. Since about 1950, many excellent papers have appeared in various languages, and one can now hope to look at the natural history of Lamarck himself. This book is a major contribution, but it suffers somewhat, like so much written recently by historians and philosophers, from a lack of biology. For example, Burkhart says that Lamarck's theory of evolutionary change was a specially eighteenth-century one, seen also in

Adam Smith, Hume, or Rousseau, in which a natural process takes place, but it is affected by constraining circumstances. The implication is that he was a child of his time, which in general is certainly true. But nevertheless he and all other biologists knew that the embryo in the uterus develops and complicates enormously in a nearly constant environment; there must therefore be a power of development independent of environmental fluctuations. He knew also that plants and animals when domesticated might change surprisingly; there must therefore also be a power of the environment over the results of development. It is difficult to see why this is a specially eighteenth-century interpretation.

Equally, there are moments when one wonders whether the author appreciates what Lamarck is referring to. Burkhart mentions Lamarck's pointing out that "the isochronous movements of the large, soft radiarians" is like the movement of the liquid in a heated thermoscope. What Lamarck is referring to is simply the pulsations (as they swim) of big jellyfish, which will be familiar to most readers. Burkhart stresses the importance of conchology, but does not give us any insight into what it means to Lamarck in his daily work and thought, apart from the example of the trigoniid already mentioned. Too much history of biology written by historians and philosophers omits too much of the biology. A history of ideas is good in itself, but in science, as against philosophy, those ideas have a practical context within the subject matter of that science which cannot be ignored. We have been shown far too often how scientists are the creatures of their times (which is true) and far too seldom how they are also influenced by their subjects. One possible source for Lamarck's ideas of fluids circulating and 'forming' vessels for themselves might be found in the teratological and embryological knowledge of the period.

The book is exceptionally well written, with only a few infelicities of translation (brute matter is not a good translation of *matiere brut*, and conscience in French is not conscience in English) or idiom (no-one did anything in 1797, only *as of* it, an unnecessary pseudolegalism) or checking (notes 88 and 89, chapter 5, and note 1, chapter 6 are incorrect references according to my copies of Lamarck). It will be an indispensable introduction to the subject, and the basis on which an examination of Lamarck's actual biological knowledge can be reared. □

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Homo exquisitus

R. D. Meikle

Ehret: Flower Painter Extraordinary.
By Gerta Calmann. Pp. 160. (Phaidon:
Oxford, 1977.) £16.

THE history of Georg Dionysius Ehret (1708–1770) exemplifies the plight of many a botanical artist, dismissed (until recently) as a technician by the world of Art, and used, but scarcely appreciated, by botanists, to whom such elegant work seems to be of little more than peripheral importance.

Ehret was born in Heidelberg (or possibly Erfurt), the son of a gardener, with no advantages of birth save an observant eye and a natural talent for drawing and painting flowers. After a brief apprenticeship in horticulture, he turned to botanical illustration, serving a succession of patrons, who, it would seem, were rather more anxious to exploit his genius than to reward it. In many ways, his life resembles that of Mozart: plenty of appreciation, and no lack of hospitality, but probably always an acute shortage of cash. Like Mozart, his strivings for a measure of independence were labelled giddiness by his betters: “he is homo exquisitus in everything, he has only one fault, he is flighty”.

His end was not a tragic one, perhaps because he had the good sense to head for England, where in time he became something of a celebrity, commending himself to Mrs Delany and the Duchess of Portland, and no doubt discovering that nothing is more acceptable to the English than an imperfect command of their language; for, even after thirty years’ residence, he could barely make himself understood. In view of his reputed flightiness, even the daunting task of teaching the daughters of the nobility how to draw and paint flowers may have had its rewarding moments.

From his arrival in England, in 1736, until his death thirty-four years later, Ehret’s life seems to have been tolerably pleasant, and was certainly very productive. It was not, however, the sort of life that was likely to contribute to his lasting fame. Much of his best work remained unpublished. Splendid paintings on paper and vellum may have given momentary gratification to the proud owners of stoves and exotica, but they were soon filed away and forgotten. The published engravings, even those in Linnaeus’s *Hortus*

Cliffortianus and his own *Plantae et Papiliones Rariores* can scarcely be judged more than competent, whereas those in Trew’s *Plantae Selectae* and *Hortus Nitidissimus* are sometimes mere travesties of the originals, almost as lifeless as the text which they accompany. Furthermore, the very works which Ehret illustrated ceased to be of more than scholarly and historic interest once the simple binomial system of plant nomenclature had been fully grasped.

Ehret was the contemporary (and avowedly the friend) of Linnaeus, but, in company with many of his patrons, he failed to appreciate the Linnean revolution in plant-naming, and continued to cling to “pre-Linnean” phrase-names long after the publication of the *Species Plantarum* (1753). It is for this reason that the writings of Miller, Aiton and even the ingenious Hill are still valued, whereas those of Trew and Weinmann were outmoded almost as soon as they left the press. Had it not been for the recent revival of interest in Ehret as an artist, there can be little doubt that his eclipse would have been total. In the unsympathetic world of taxonomy, he is, even now, little more than a penumbra.

Dr Gerta Calmann’s monograph should dispel some of the shadows. But Ehret is a difficult person to write about. He was forever on the move, flitting, like one of his butterflies, from flower to flower and from patron to patron, without leaving any record of his activities save numerous signed and dated pictures and a brief autobiographical sketch specially designed to ease his entry into the Imperial German Academy of Naturalists. To make sense of such a complex and disjointed career, it is necessary to plan carefully and prune rigorously. Unfortunately,



Engraving by Haid after drawings by Ehret and Heckell

Dr Calmann seems to have caught some of the flightiness from her subject. The text is widely informative, and often entertaining, but however much one may be amused by the antics of the Marcgrave of Baden-Durlach and his troop of female hussars, these, and similar digressions, do not help to concentrate one’s mind on the essentials. Moreover, the author’s frank admission that she is not a botanist does not excuse the astonishing string of errors on p48. Here, we are told that, “In a flash of insight Linnaeus realised that stamens and pistils were the sexual organs of plants”, that his “artificial” system “was superseded finally by Darwin’s theory of evolution”, and that Linnaeus “created botanical Latin which simplified the identification of plants”. Such *obiter dicta* do not necessarily impair the validity of the remaining text; but they undermine one’s confidence.

The coloured illustrations, despite some, possibly unavoidable, reduction, are generally good, certainly very much better than many of the reproductions which appeared in Ehret’s own lifetime. They have been selected to span almost the whole of his career; and although they do not demonstrate any remarkable development in skill or style, they do, fairly if rather unkindly, show the artist at his best and at his worst. His quality is certainly uneven, some of the plates, especially the Long-leaf Pine (pl.46) and the Christmas Rose (pl.95) being models of freshness and grace, whereas others, notably the Rose (pl.7) and more particularly the Poker-plant (pl.54) are so wooden and opaque that it is hard to believe they are by the same hand. The flattened, dive-bombing butterflies which appear in so many of his pictures are not, to my mind, an adornment; out of charity, one hopes they were forced on the artist to satisfy the catholic tastes of his naturalist patrons.

The monochromes are on the whole less satisfactory. Those which are allowed a full page are acceptable enough, though even here the “Characters of Flowers” (pl.75) with its amusing reminder of Ehret’s faulty English, lacks much of the crisp quaintness of the original. The quarter- and half-page monochromes lose so much through reduction and absence of colour that one wonders if their replacement by a few additional colour plates would not have been wiser. The obscurity of one of these little illustrations (pl.16) may explain the misidentification of Thorow-wax (*Bupleurum rotundifolium*) as Yellow-wort (*Blackstonia perfoliata*). □

R. D. Meikle is on the staff of the Herbarium at the Royal Botanic Gardens, Kew, UK.

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South African cheer

K. L. Manchester

A History of Scientific Endeavour in South Africa. (A collection of essays published on the occasion of the Centenary of the Royal Society of South Africa.) Edited by A. C. Brown. Pp. 516. (Royal Society of South Africa, c/o University of Cape Town, 1977.)

ON 22 June 1877 in Cape Town was founded the South African Philosophical Society, which on 25 January 1908 by grant of Royal Charter from Edward VII became the Royal Society of South Africa. *A History of Scientific Endeavour in South Africa* has been written to commemorate the centenary of the Society, which is the oldest body of its kind in the country—probably in the African continent as a whole. Edited by A. C. Brown, Professor of Marine Biology at the University of Cape Town, the book consists of a series of historical essays by some of South Africa's leading scientists, recording the efforts of workers in many fields who have broadened knowledge and understanding of the South African habitat.

The Foreword by the State President describes the book as a tribute to the many who have contributed much of the basic knowledge and research that has made possible the transformation of South Africa from the remote and little-known land of hunters, pastoralists and subsistence agriculturalists it was in the seventeenth century to the economically advanced nation that has emerged in the twentieth—one of the world's leading producers of many minerals and the leading industrial nation in Africa. This may all sound a little like advertiser's copy, but as one of the contributors to the volume concludes: "It is doubtful whether many other countries can show a similar growth of scientific institutions and of the solid body of original work carried out by them, if one takes into account the size of the population."

Of course, South Africa has in some ways had things made for it. The enormous mineral deposits have been provided by Nature, even if exploited by man. The clear skies have attracted the astronomers and the wealth of wildlife the biologists. Not only is the wildlife extremely rich and varied but many of the species are endemic, due to the relative long isolation of parts of South Africa by desert from the rest of the continent. It is interesting to read that even in the early 1800s there was a

considerable demand for South African wild animals in Europe, and their export, both dead and alive, was a profitable business. Gone are the days when the springbok trekked over the Karoo in vast herds to the delight of the hunter. Wildlife conservation is rather better arranged today, and the game reserves of South Africa must rank amongst the best managed in the world.

More academically, in *Australopithecus africanus*, South Africa has fulfilled Darwin's prophecy that Africa would prove to be the home of the 'missing' link, and lately evidence of the presence of the more advanced *Homo habilis* has come to hand. The Karoo Formation is also remarkable for its wealth of unique fossil reptiles and the east coast waters for the coelacanth.

Inevitably, many of the chapters read like a catalogue of names and events which for the average reader will prove difficult to assimilate. Nonetheless it is impossible not to perceive the steady growth through the years of the various scientific disciplines from the incredibly primitive to the sophistication of the present day. The early settlers suffered grievous losses of crops and stock due to unfamiliar pests and disease. Pains-taking veterinary and agricultural research has overcome many of these problems, vastly extending the extent of habitable country. Institutions such as Onderstepoort, built up by the redoubtable Theiler, have a worldwide reputation.

Proper medical education in South Africa only began in 1912. Yet today, the Medical Schools of Cape Town and the University of the Witwatersrand stand amongst the best. *Xenopus laevis*, the South African clawed toad, has made its mark in modern molecular biology; but to a wider public the discovery in Cape Town in 1933 that *Xenopus* could be used for a bioassay for human pregnancy has possibly been of greater significance.

The exploitation of mineral resources naturally has stimulated engineering and metallurgical research. Rockbursts have to be understood if they are to be avoided. Pursuit of the gold bearing reefs to ever increasing depths has given South Africa unique experience of the engineering problems of deep level mining and the health hazards involved. Mining is a great consumer of energy, but South Africa is blessed with 60% or more of all African coal resources; and neighbouring territories have considerable hydroelectrical potential. We may no longer dream of a Cape to Cairo rail link, but electrical reticulation of southern Africa is today of considerable logistic significance. Lack of oil is the Achilles heel of the South African economy. In the SASOL

coal to oil process the country seeks self-sufficiency both for oil and petrochemicals.

A final chapter is devoted to the history of the Royal Society itself. It is amusing to find that in 1902 and 1905 the Society made a strong case for metrication of weights, measures and coinage in South Africa, an attempt that failed due to pressure exerted in the British Parliament. The Society was 60 years too early.

It is impossible to summarise in any meaningful way the mass of detailed material contained in this volume. To

anyone with an interest in South Africa or in the historical concept of how a country can develop scientifically the book is a must. The Royal Society of South Africa is to be congratulated in seeking to put together for the first time the fascinating story of the growth and discoveries of science in southern Africa. Into an atmosphere of political gloom and frustration it injects a note of human achievement and cheer. □

K. L. Manchester is Professor of Biochemistry at the University of the Witwatersrand, Johannesburg, South Africa.

Historical revisionism

M. J. S. Rudwick

The Making of Geology: Earth Science in Britain, 1660–1815. By Roy Porter. Pp. 288 (Cambridge University: London, Cambridge and New York, 1977.) £8.95.

MOST Earth scientists are conscious of the great conceptual integration that plate tectonic theory has brought to their science in the past two decades. In retrospect, the immediately preceding period seems like a time of relative stagnation or, at best, of several disparate disciplines evolving in relative isolation.

This situation is not unlike an earlier period, to which historically conscious geologists often look back to find the origins of Earth science as a whole. In doing so, however, they generally adopt an historical interpretation that was itself a product of that period. The scientists who first gave 'geology' its institutional and cognitive identity in the early nineteenth century looked back to the eighteenth and late seventeenth centuries as a period of benighted obscurantism or fanciful speculation, relieved only by a few isolated

pioneers or forerunners of their own approach.

This is the interpretation that Dr Porter has set out to demythologise. His book is a conscious piece of historical revisionism, yet in no sense is it a facile exercise in debunking. His starting point is the striking discrepancy between the negative evaluation that nineteenth-century geologists gave to the work of their predecessors, and the actual content and scope of that work. The latter is reconstructed by historical analysis of an impressive array of published books and articles, unpublished field notebooks and correspondence, and other material, illustrated by often fascinating quotations. Dr Porter suggests, in my opinion persuasively, that early nineteenth-century geologists rejected or disowned the past history of their subject, because they felt they had successfully constructed a self-sustaining social enterprise with a new research programme of unlimited potentialities—significantly, the very word 'geology' only came into general use at that time. They contrasted this with the relatively undirected and even confused activities of their predecessors. Failing to see how these activities had been an indispensable precondition for their own work, they exaggerated the contrast into an almost total discontinuity.

Making is the key word in Dr Porter's title and in his interpretation.

Although the Earth was in an obvious sense always 'there' to be studied, the body of theoretical ideas and practical techniques that characterised the new science of geology was constructed out of the choices and decisions of individuals and groups in specific historical circumstances. To put it another way, the manifest heuristic success and cumulative character of the mature science of geology since the early nineteenth century is a historical phenomenon that calls for explanation, just as much as the 'failure' of earlier generations to create such a self-sustaining enterprise.

Dr Porter's approach is one in which the traditional dichotomy between 'internalist' and 'externalist' history of science is shown up in all its sterility. It is pointless to trace the growth of a community of like-minded 'geologists', or the reasons why that group developed when and how it did, without analysing the theories and techniques to which their likemindedness was directed; but the converse—the style of traditional 'internalist' history of science—is equally futile. Dr Porter succeeds admirably in blending context and content into a single integrated interpretation.

This book is not, and does not claim to be, a definitive or exhaustive description of all the research that was carried out during the period 1660–1815, even within Britain. It is a suggestive outline of a new way of looking at the early history of the earth sciences. The first appearance of a self-conscious science of geology is not seen as a product of an intrinsic progressiveness of knowledge or of the genius of a few pioneers—interestingly, the Scottish natural philosopher, James Hutton, still sometimes called 'the founder of modern geology', is shown to have been somewhat isolated from the mainstream development of the science. Instead, the emphasis is on the purposeful construction of an integrated discipline with clearly defined methods and—at first—quite narrowly limited cognitive aims; and this construction is seen as the work of individuals and groups working in specific circumstances that constrained the social forms in which their activity was embodied.

The geology that had thus been 'made' by the early nineteenth century is recognisably continuous with the earth science of today, even after its most recent 'revolution'. Dr Porter's account of this construction can be warmly recommended to all Earth scientists who are interested in the foundations of their science. □

M. J. S. Rudwick is Professor of the History of Science at the Free University, Amsterdam, The Netherlands.

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Philosophical conflict

Peter Winch

Wittgenstein and Scientific Knowledge: A Sociological Perspective. By Derek L. Phillips. Pp. 248 (Macmillan: London, 1977.) £10.

THE aim of Professor Phillips' book is to bring Ludwig Wittgenstein's philosophical work to the attention of sociologists, in the belief that it has something to contribute to issues which exercise them. It is concerned mainly with certain issues in the general philosophy of science which have recently come to the fore in the conflict between the hitherto dominant positivist and Popperian conceptions of science and the work of such writers as Thomas Kuhn and Paul Feyerabend. These issues touch the work of sociologists most directly in the area of 'sociology of knowledge', stemming from Karl Mannheim. As Phillips observes and brings out, many of Wittgenstein's ideas are very germane to such issues: particularly, the notes he wrote towards the end of his life published in *On Certainty*. The two issues in the philosophy of science and sociology of knowledge on which the book under review focusses most directly are: (1) relativism and the claim, arising especially out of Kuhn's work, that theories separated by a 'scientific revolution' are incommensurable; (2) where does authority lie in science: who, that is, decides what is respectable science and what isn't? Phillips has interesting things to say about both these topics, especially the first, though his treatments of them are not entirely consistent with each other. But I shall return to this point later.

The structure of the book is loose and not altogether perspicuous. There are chapters which are exclusively expository of some of Wittgenstein's main philosophical ideas; chapters in which some of these ideas are brought to bear directly on those issues in the sociology of knowledge in which Phillips is most interested; a chapter in which those issues are treated without explicit reference to the relevance of Wittgenstein's work to them. Overall, this gives an impression of disjointedness: the various issues discussed do indeed have important interconnections, but they are too often presented in indigestible chunks, and not enough work has been done to work them into a coherent whole. This is especially true of the

relationship between the first chapter on 'Wittgenstein the Man' and the exposition of his philosophy.

Phillips touches on an important and difficult tangle of problems when he claims that there is an "intimate link between [Wittgenstein's] life and his work", but he does not really attempt to clarify them. He relies very uncritically on sources concerning Wittgenstein's life which are less than impressive, such as W. W. Bartley III's *Wittgenstein*, some of whose extravagant claims are accepted as gospel, without any allusion to the surely justified doubts of many reviewers of that book about the quality of the evidence adduced in support of those claims. More importantly perhaps, he shows no signs of having reflected on any of the really puzzling questions about the relationship between a man's life and his work of the sort raised for instance, by Rush Rhees in his discussion of Bartley's book in *The Human World*. Yet those questions, one might think, have a direct bearing on the issues in the 'sociology of knowledge' which are at the centre of Phillips' interest.

I am not sure how useful the very compressed account of Wittgenstein's early and later philosophy will be to those to whom it is addressed. I doubt if it will be very intelligible to people who are not already pretty familiar with the issues involved and, though the presentation is superficially lucid, there are a number of more or less serious examples of carelessness of which the following are just examples. On page 30 it is said that, according to Wittgenstein's *Philosophical Investigations*, "Nothing exists outside of our language and actions which can be used to justify, for example, a statement's

truth or falsity". It is true that Wittgenstein opposed the view that there need or could be an "external" justification of the grammar of our language; and also that he thought this grammar required a certain "agreement in judgements"; but this does not mean that he denied the possibility of establishing the truth or falsity of *any* statement by reference to things which exist independently of us and our linguistic practices. Again, Phillips writes, on page 82, that according to Wittgenstein "it is a contingent fact that the concepts of pain and grief connect up with a certain uniformity of judgement among human beings". Clarity about what is contingent and what is not is of the essence in such issues: what Wittgenstein said is contingent is that there should exist the sort of "uniformity of judgement" among human beings which makes it possible for them to have concepts such as grief and pain; *not* that, given such uniformity of judgement, men's concepts of grief and pain connect up with it. I am afraid that slips of this sort, about quite fundamental issues, may seriously mislead the newcomers to Wittgenstein's philosophy to whom, I take it, the book is addressed.

Chapter 5 seems to me the best. It deals with claims about the incommensurability of scientific theories involving different "paradigms" arising out of the work of writers like Kuhn. Here, Phillips makes illuminating use of Wittgenstein's view that the language of scientific theorising has a continuing dependence on the language of everyday life; and he connects this usefully with Wittgenstein's discussion of the distinction between "seeing" and "seeing as" (or "seeing an aspect"). This chapter deserves attention by anyone interested in the questions surrounding the notion of incommensurability of theories. I hope readers will not be misled by Phillips' idiosyncratic use of the term "meta-language" in speaking of the relationship between the language of everyday life and the languages of the sciences. His conception has nothing in common with that expressed by the established technical use of "meta-language" amongst philosophers of language and logicians influenced by Tarski.

Chapter 7 has some useful material on the power structures of scientific institutions and their role in the "validation" of scientific theories. Phillips surely goes too far, however, when he claims that the individual scientist's "explanatory achievements are *fully* [my emphasis] dependent upon the judgements of the scientific community or the speciality area in which he works" (page 40). This would lead back



Wittgenstein

Courtesy Master and Fellows, Trinity College, Cambridge

to the type of relativism which earlier chapters were designed to avoid; and Phillips here forgets his own correct contention that the technical languages spoken by "scientific communities" are not fully autonomous, but depend for their sense on a relationship to the non-

technical language used in the wider human community to which they belong. A particular scientific elite is not a court beyond which there is no appeal. □

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Remote and legendary figure

Sydney Selwyn

Joseph Lister, 1827-1912. By Richard B. Fisher. Pp. 351+16 plates. (Macdonald and Jane's: London, 1977.) £7.95.

ON the 150th anniversary of his birth and a mere 65 years after his death, Joseph Lister has already become an extremely remote, almost legendary figure. Even in his lifetime, during the 40 years of fame when every conceivable honour came his way, Lister appeared aloof and enigmatic. Little or no insight into his personality can be gained from the only two full-length biographies available until now, that of G. T. Wrench published in 1913, and the official one by Lister's nephew, Sir Rickman Godlee, which first appeared in 1917. Happily, the new biography by Richard Fisher deals fully not only with Lister's varied scientific and surgical work, viewed from the perspective of our own time, but also with Lister the man. Drawing on a considerable volume of unpublished correspondence, diaries and laboratory notebooks, the author's portrait will reveal for most readers an unexpectedly complex individual. Certainly, the popular view of Lister as the serene benefactor of mankind whose work on antiseptic surgery was greeted with universal acclaim has long needed correction. A glance through the editorial and correspondence columns of the *Lancet* and *British Medical Journal* from 1867 into the 1880s shows Lister to be at the centre of polemics—many of his own making and not all concerned with antiseptic surgery.

Lister was fortunate in his background—a congenial quaker family originating in Yorkshire, quaker friends such as Thomas Hodgkin and Elizabeth Fry who were devoted to science and philanthropy, and a prosperous wine merchant father, Joseph Jackson Lister, who by 1830 had made fundamental contributions to microscopy and was to become a life-long mentor and confidant of his son. As a student Lister performed extremely well at University

College, London until 1847, when he underwent a religious crisis and in March 1848 suffered a severe 'nervous breakdown' of a depressive nature, which prevented any further work for 18 months. Although he eventually made a good recovery, Lister subsequently was afflicted by recurrent self-doubts and a type of paranoia. His biographer convincingly uses psychological analysis to explain the paradox of a man who was gentle, kind, chivalrous and in many ways noble, but who was often publicly tactless, obtuse and, for example, an implacable opponent of the entry of women into medicine. Fortunately, Lister's mar-



Lister at Glasgow in 1865

riage, though childless, was extremely happy. His wife (a non-quaker) was his constant support, amanuensis and laboratory assistant. Moreover, in the stormy early days when Lister was under frequent attack in the medical journals he had the consolation of regular support from the young journal *Nature*.

Lister's scientific work is, in general, well reviewed. The early studies on involuntary muscle, inflammation and blood coagulation earned him, at the age of 33, a Fellowship of the Royal Society—where he joined his father and was eventually a most distinguished President. Most of this work was primarily carried out to provide material for Lister's undergraduate lectures. Using the microscope perfected by his father, Lister over a

ten-year period made meticulous observations on various contractile tissues and on the effects of irritants on the microcirculation of the frog's foot web. He greatly clarified the early events in inflammation by describing, in sequence, arteriolar constriction, slowing of blood flow and increased adhesiveness of erythrocytes—the latter having been first observed *in vitro* by his father and Thomas Hodgkin in 1827. Simultaneous changes in the pigmentation of the foot web underlined for Lister the truth of John Hunter's belief that inflammation was an active process. Similarly, blood coagulation was also shown to be a series of processes governed by tissue and vascular factors and not due, as was widely believed, to the passive loss of a 'liquefier' such as blood ammonia.

Lister always held that this work was his major scientific contribution: bacteria, which were so prominent in his later work, were simply specific irritants that triggered off inflammation and, occasionally, intravascular coagulation. To most modern observers, however, his pioneer microbiological researches from 1868 until the mid-1880s seem far more important. They were not only crucial in the battle against abiogenesis, but provided much-needed scientific support for Lister's 'Antiseptic System', which was at first extremely vulnerable at the hands of informed opponents.

Adequate attention is given in the book to Lister's technical achievements, including his method of obtaining in 1877 a pure bacterial culture by serial dilution. But the author in discussing the complex early studies on bacteria and fungi misses the extraordinary fact that Lister observed the antibacterial action of *Penicillium* species in 1871, and later used crude penicillin preparations in treatment.

Unfortunately, the book contains many medical and medical historical solecisms. For instance, jaundice is not synonymous with hepatitis, pin-worms do not cause skin disease ('ringworm' does), hospital gangrene was not due to soil-borne bacteria, and hydrocele is not a testicular growth. William Harvey published in the seventeenth, not the sixteenth century; Sir James Simpson worked in Edinburgh, not Aberdeen; Ehrlich's great chemotherapeutic work was on arsenicals, not mercurials; and Behring was certainly not a colleague of Pasteur. Although these and other blunders are irritating, Mr Fisher has written a definitive biography of a remarkable man. Such a book has been long awaited and is to be warmly welcomed. □

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American Nobel elite

Steven Rose

Scientific Elite: Nobel Laureates in the United States. By Harriet Zuckerman. Pp. xv+335. (Free Press; New York; Collier-Macmillan; West Drayton, UK, 1977.) £11.25.

NOBEL PRIZES are news and not merely because the money is of the order of a middle-sized pools win. The awards make not only *The Times* but the BBC news and *The Mirror* as well. National statistics of Nobel Prize winners are counted anxiously as a measure of scientific health. The Prize is seen as the apotheosis of a scientist's career, and the equality of opportunity with which graduate scientists are supposed to start up the hierarchical ladder emphasises the myth that, like napoleonic field-m Marshals, the laurels are in every Ph.D. student's lab-coat.

Nobel Prize winners are the nearest equivalent in science to archbishops, or pop or movie stars (barring the age-differential, of course, so far as the latter are concerned). They are invited

to serve on government committees indifferently, more-or-less, to the actual subject-matter of their expertise; the media hang on their words; films and TV plays are made about them. They pronounce with authority on subjects as diverse as ethics and ecology, the limits of human intellect and of industrial growth.

No appeal for humanitarian causes, whether a paid advertisement in the western press or a smuggled *samizdat* letter, is complete without the mystic *imprimatur*. Clearly, the phenomenon of the laureate is one of some importance in understanding the social system of science, whether considered from the point of view of the internal 'community' of scientists themselves or its relationship to the wider social matrix within which it is embedded and which it helps shape.

For the sociologist, I would guess that the problem of the study of elite groups must be considerable. Their numbers are small and individuals easily identified so that generalisations are harder; they are likely to be less amenable to standard procedures of interview or questionnaire than the less illustrious subjects who generally form the core of sociological specimens, and this must put the researcher's relationship with his or her subjects on a different (more deferential?) footing. Also, the objects of one's survey are actually likely to read what one writes—and if they don't like it, they have their ways. . . . Perhaps it is for this reason that the poor are more often studied than the rich?

Despite these problems, some of which she discusses, Harriet Zuckerman has been attempting, since the early 1960s, a dissection of the anatomy of the Nobel elite, or at least that not inconsiderable fraction of it which is resident in, and preferably born in, the US. She began by interviewing some 41 of the 56 laureates alive in the US in the early 1960s, and has subsequently extended her survey backwards to the first US laureate, and sideways to holders of what she calls the "41st chair"—scientists whose work is rated as of "Nobel quality" but, for varying reasons, were never awarded the Prize. Through some 200 pages of her book this tiny group are tabulated and cross-tabulated.

We learn that American laureates are more likely than the average researcher to come from an upper-class background (rating below supreme court justices, but above admirals and generals); more likely to be of Jewish and less likely to be of Catholic parentage (for complex reasons); more likely to have gone to elite colleges as undergraduates and especially as Ph.D.

students; and quite likely to have been chosen by, or opted to work with, a present or future laureate early in their career. They have written more papers, more often cited, than the generality of scientists or even other lesser-prestige groups, such as NAS members. They were promoted earlier to Chairs. They are likely to have done their prize-winning work at around 39 (older for the physiology or medicine prize, younger for the physics one) but only get the Prize some 12 years later. Half of the prizes have been won for work carried out in five US institutions.

Once they have received the Prize, their productivity tends to decline, but still remains high compared with other scientists. They accumulate many more honorific awards; they help construct new laureates; as they grow older, many turn towards philosophy.

There are a number of possible reactions to this careful compilation of data; for many professional scientists, it is likely to be an unsurprised "so what?", as they see so many of their rule-of-thumb categorisations confirmed, coupled with an enjoyable guessing game as they try to assign the unattributed quotes with which the Zuckerman text is enlivened. For the empirical sociologist of science, it is pleasing to see such a pretty working out of what Zuckerman's mentor, R. K. Merton, has called "The Matthew effect" in science—to him that hath, more shall be given; a maxim which clearly applies both to impending laureates and the institutions in which they work.

In addition, by showing convincingly that the Nobel award is neither a pure accolade of merit or part of a self-fulfilling prophesy, but is a combination of achieved and ascribed glory, Zuckerman has gone a little way towards demystifying the Prize. Laureates are neither born nor made, but are produced by one of those well known but little understood interactions. She has thrown up some further empirical 'puzzles', such as that of the religious background of laureates, and provided a basis for the comparative study of scientific and other elites (for instance, between science and literature Nobel laureates?).

One cannot help feeling, however, that this type of approach leaves many of the central questions untouched. The Prize glorifies the individual scientist, yet in an era of big science, where production, particularly of physics, has become virtually industrialised, most research has become teamwork. Teams cannot win Prizes—except, it seems, for peace. So, either they are unrewarded or some of their members are singled out—theoreticians rather than experimentalists, generally; professors

Scientists under Hitler

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Alan D. Beyerchen

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rather than graduate students, generally; men rather than women, generally; qualified scientists rather than the technicians on whom their work depends, always.

The Prize typifies the myth of 'science' and obscures its reality. It emphasises the hierarchical and authoritarian shape which science in the late twentieth century has come to take. Its social functions inside science and in terms of the relationships between science and society, are both symbolic and substantive, and proper

matters, one would have thought, for a critical sociologist to discuss; yet in all of Zuckerman's pages scarcely a mention is made of such questions. Indeed, it has so far been left largely to scientists themselves to voice such doubts. It would be nice if, now she has done such a thorough piece of internalist empiricism, she would move on to these richer and more complex questions. □

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Dazzling firmament

Rainer Goldsmith

The Pursuit of Nature. By A. L. Hodgkin, A. F. Huxley, W. Feldberg, W. A. H. Rushton, R. A. Gregory and R. A. McCance. Pp. 180. (Cambridge University: London, New York and Cambridge, 1977.) £7.50.

PITY the poor reviewer, a mere mortal, confronted with a book of essays the authors of which are the living immortals of his discipline. Praise will be interpreted as sycophancy; criticism is tantamount to lèse-majesté. Yet the task must be done, and this fool will step in where wiser angels fear to tread.

The six essays, collectively entitled "Informal Essays on the History of Physiology" are mixed bags containing reminiscences, anecdotes, historical analyses, futuristic speculation, straightforward review, and personal prejudice. They make for varied reading; hardly ever boring, in part thought-provoking and often leaving the reader gasping with admiration at past achievements.

Outstanding among the six is the contribution of Feldberg, "Reminiscences of an Eye-Witness", being an account of the discovery and confirmation of the nature of synaptic and neuromuscular transmission. The lively and very personal style of the essay graphically reflects the enthusiasm and excitement of two or three highly creative years, interspersed by lobster dinners to celebrate successful new findings. One may wonder, however, if Feldberg really contributed only the key of eserine and the eserinated leech muscle to this remarkable period in physiology. Twenty-five years after the event, your reviewer began his scientific career in the very room in which these momentous advances were made: the ceiling was crumbling, the teak benches were uncommunicative witnesses of their past glory—just in one cupboard did the ghosts still linger, the shelves were marked Dale, Feldberg, Brown and McIntosh.

In general, the other essays are also best when the authors write about their own experiences or their immediate interests. The last quarter of Rushton's essay, the piece on colour vision, the *femme fatale* by whom he is so willingly seduced, is entertaining, informative and provocative, indicating that discoveries still await the lucky or the hardworking. The rest, though not up to the same standard, can hardly fail to please. Gregory's vivid account of a single afternoon's work which led to the discovery of secretin and the frustrating tale of the discovery, rejection and rediscovery of gastrin, are clearly very near to his heart.

McCance's contribution is a staccato, somewhat disjointed, review of perinatal physiology interspersed with acid comments on political matters, climaxed by an attack on ethical committees "which shackle the human physiologist to a politically-minded and usually uncooperative public". A

clearer explanation of what the physiologist is about would bring about greater cooperation.

What of the two brightest stars in this dazzling firmament?, the Nobel laureates: your reviewer is now skating on very thin ice. Sir Alan Hodgkin gives a dry, low-key, understated account of his own and other experiments which led to the explanation of the nature of the action potential. His picture of Cambridge in the 1930s is that of another world. But, with decreasing funds, we too may have "to think hard to decide what is right" and then "start in a bare room and build most of our equipment". The writing lacks Feldberg's sense of immediacy and involvement; it seems that life was an orderly procession from Cambridge to New York and the grand tour of the United States with just time for a less-than-one-dollar-a-day holiday in Mexico and then back to Cambridge and Plymouth. Great events are curiously muted: the tribulations of war are recalled by the fact that he did not bother to keep his copies of the *Journal of Physiology*, their alleviation by marriage to Peyton Rous' daughter, and his ability to think about nerves again; the 1947 fuel crisis during which all Cambridge and indeed the rest of the country froze, is marked only by the chance to do experiments at -4°C . It is an account of a dedicated scientist hard at work undisturbed by counter-attractions—how unlike the more frivolous attitudes of a younger generation of Cambridge laureates (see J. D. Watson, *Double Helix*, Weidenfeld and Nicolson).

"Looking Back on Muscle" by A. F. Huxley is a masterly account of the changing views down the microscope over the past hundred years, which lead eventually to the sliding filament theory of the contraction of muscle. It is a stern warning that what is true today may be forgotten tomorrow and that yesterday's discoveries may be nearer the truth than today's dogma.

All in all, a stimulating book requiring a good physiological background to obtain full value. It is a pity that these eminent men allude to so much endowing us with their own immense understanding but in fact leaving most stranded on the shores of ignorance. It is as if the secrets must remain hidden from all save those who have been fully initiated into the circle. It is an enjoyable book for the student, opening the door into living history and introducing him to all the greats, not only these six but also their colleagues and their teachers who constitute the very backbone of physiology. □

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cities that are brought on by experience. He rejects "the fantastic notion that every memory is innately within us", but his own evidence is consistent with the conception behind my mnemonic hypothesis (which he quotes) that memory is a selective process, involving loss of some channels and improvement of others. Like all good neuroscientists he accepts that neural coding involves putting each item of information into a separate channel, but we all find it difficult to work out the im-

plications of this multichannel method of operating even for simple acts of perception.

To be able to discuss such questions in a review of the book of a series of broadcast lectures that are widely listened to is the highest tribute one can give to the author and to the BBC. Can any other country equal the record of this series? ☐

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Just not cricket

Stuart Sutherland

Against Behaviouralism: A Critique of Behavioural Science. By Edmund Ions. Pp. xiii+165. (Blackwell: Oxford, 1977.) Hardback £6; paperback £2.75.

THE sport of debunking famous names rarely fails to entertain, and when applied to men as pompous and pretentious as most social scientists it has enhanced spectator appeal. As a demon bowler Edmund Ions is in a class of his own: he dismisses in successive balls Herbert Simon, Morton Kaplan, Karl Deutsch, B. F. Skinner, Rashevsky, Stouffer, Adorno, von Neumann, Kaldor, Hicks, Garfinkel, Goffman, Levi-Strauss and Chomsky. His opponents are a curiously assorted team: were it not for the inclusion of Garfinkel and Goffman one might suppose that Ions was combating the attempt to apply objective methods in the social sciences, but ethnomethodologists can scarcely be accused of that and are declared out for giving "a partial and selective account of human behaviour".

Against Behaviouralism is primarily an attack on the use of mathematical and logical theories, and of scientific methods of data collection and analysis in the social sciences. Ions argues his case by examining a series of instances where the attempt to be scientific has resulted in banal or useless theories, or where objective methods of data collection have been poorly applied and grossly misinterpreted.

There is plenty of bad work in the social sciences and many of Ions' examples are convincing. Grandiose models of the sort constructed by Rashevsky are empty; cost-benefit analysis invariably involves making arbitrary assumptions; the celebrated discovery of the "Authoritarian Personality" rests on the construction of a loaded questionnaire and the use of a monstrously biased sample; Games Theory is an idealised model of conflict

behaviour; and cliometricians, jurimetricians, psephologists and content analysts often either discover the obvious or make wild errors as a result of unduly restricting both the data they use and their methods of analysis.

Moreover, Ions is surely right in thinking that the very obscurity of many scientific approaches to the social sciences has disguised the fact that much of the work is empty. Model builders often conceal the banality of their thought by the tortuousness of their language. It requires both perspicacity and courage to dismiss as trivial such statements as: "*Hypothesis Two*. Multiple role functions operate on personality systems in such a fashion that the personality systems perceive the objective and values of the decision making units linked by the multiple role functions as more similar than they would if they entered only into single role function." (Morton Kaplan.)

We may agree with Ions, then, that the application of scientific or objective methods is no guarantee of producing interesting findings or arriving at correct conclusions, but he seems to go beyond this and to damn all attempts at objectivity. Yet, the use of intuition can be just as fallible as is demonstrated by the results of studies on the unreliability of unstructured interviews for job selection. One only has to read Arthur Maslow to become aware that the experiential or humanist approach to society may itself result in pretentious banality and silliness. And even if rigorous models are as incomplete as non-rigorous ones, they may still throw light on human behaviour by focusing attention on the ways in which people depart from them.

Ions stigmatises Levi-Strauss for "classifying", though how it is possible to communicate at all without classifying he does not explain. His critique of Chomsky is hard to follow. He seems to think that the attempt to formalise our intuitions about syntax is in itself a bad thing and then goes on to criticise Chomsky for failing to deal with those aspects of language that are completely outside the scope of his theories.

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A further problem is that in arguing from example, Ions is himself guilty of one of the main crimes of which he seeks to convict his opponents, namely, dogmatic generalisation based on insufficient evidence. He maintains a consistently fast pace but his bowling is loose and often aimed at the man

not the wicket: any decent umpire would have sent him off the field, but that would have deprived the crowd of considerable amusement. □

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Animal communication in ethological research

John Krebs

The Behaviour of Communicating: An Ethological Approach. By W. John Smith. Pp. 545. (Harvard University: Cambridge, Massachusetts and London, 1977.) £13.65.

MORE than any other subject, animal communication has occupied a prominent place in ethological research throughout the past forty years. Lorenz's demonstration that displays of ducks and geese can be used as species-specific taxonomic characters to trace phylogenies; Tinbergen's classic analyses of the motivational basis of displays in gulls; Thorpe and Marler's work on the ontogeny of bird song; and von Frisch's dancing bees are all justifiably recognised as pillars of ethology. In the past few years, although some of the emphasis has slightly changed, animal communication has still played a central role in ethological thinking, exemplified by G. A. Parker and J. Maynard Smith's application of game theory to ritualised displays.

It is perhaps not surprising, in view of the voluminous and diverse literature, that no single person has, since Tinbergen with his *Social Behaviour in Animals* (Methuen, 1953), attempted the daunting task of reviewing the whole subject of communication. There have been notable collections of essays (Huxley's "ritualisation" symposium, Hinde's *Non-Verbal Communication* and Sebeok's *Animal Communication*), but W. John Smith is the first for nearly 25 years to write a comprehensive treatise. Comprehensive is the word: with over forty pages of references, Smith has done an admirable and scholarly job of bringing together the literature up to and including 1976.

Smith's own well known contribu-

tion to the field of communication has been to emphasise the distinction between the *message* contained in a signal (what it might potentially reveal about the communicator) and its *meaning* to the recipient(s). The meaning, measured by the response of the recipient, usually depends on the context. For example, a hypothetical call given by a bird whenever it moves may mean "I am about to attack" in one context, and "I am about to feed" in another. The book leans heavily on Smith's message-meaning distinction: after an introduction in which he clearly defines communication and states the problems he is going to tackle, there are six chapters (including a lot of descriptive examples) dealing with the structure and message content of signals.

The subsequent chapters deal in turn with the motivational basis of displays (a review of the traditional ethological work), the importance of context and the meaning of signals, ritualisation, ecological and other constraints on signalling systems, and finally a critique and reassessment of the display concept. In this final section, Smith points out that the usual preoccupation of ethologists with simple stereotyped displays (releasers) has led to an underemphasis on the possibility of complex grammatical rules for combining simple components into complex signals. Throughout the book, Smith provides numerous examples, many of them unpublished, which make interesting reading and act as an excellent diluent for the slightly heavy style of the more theoretical discussions.

In such a wide-ranging and thought-provoking book, it is hard to know where to begin in singling out points for discussion, but I will mention briefly a general feature of communication which I believe that Smith may have underplayed. Smith views communication as a cooperative venture between communicator and recipient; displays are acts "specialised to make information available to the recipient" (p69, p195) about the communicator's likely future behaviour, status, location, and so on. This standpoint is emphasised by Smith's statement that manipulation or intentional misleading is a "disturbing possibility" but not one that ethologists have yet discovered. (He inexplicably separates intra- and interspecific deceit—the

latter is well known in, for example, Batesian mimicry.)

I would suggest that far from being a marginally possible aberrance, manipulation, or something very like it, is a central feature of much of animal communication. As Smith emphasises, signals can only evolve if there is an advantage to the recipient in responding, as well as an advantage to the communicator. But this is not to say that the benefits to the two are equal; and it seems inevitable that both participants in any interaction—be it between a territorial stickleback displaying to an intruder or a fledgling blackbird begging from its parent—will strive to get the maximum benefit. Viewed in this light, communicators are always trying to manipulate or persuade recipients, while recipients are increasing their sales-resistance. It is then not at all surprising that displays often provide incomplete information about the motivational state of the communicator (p201): it always pays to be poker-faced when selling a used car.

It also follows from my argument that many display interactions are concerned with assessment ("Is he really as confident as his threat indicates?"). The elaborate courtship rituals of many animals may have as much to do with assessment of the potential mate's fidelity and fecundity as with "helping male and female to cooperate in beginning copulation" (p300). This was brought home to me by the striking result of Erickson and Zenone (*Science*, **192**, 1353–54; 1976). They showed that a male ring dove actually rejects a female who is too willing in courtship. This is a canny reaction: "precocious" females are forward because they have just been stimulated by mating with another male, and it supports the view that ring dove courtship involves assessment by the male of whether the female has already been fertilised. With the traditional ethological view of male courtship as a means of arousing the female to copulate, who would have foreseen that male ring doves would reject avian nymphomaniacs?

While I certainly would not claim that all intraspecific communication involves manipulation and assessment, it just might be useful to bear these ideas in mind when analysing courtship and threat signals. Finally, let me emphasise that Smith's omission of the sort of discussion I have briefly outlined does not materially detract from his enormous achievement in synthesising and appraising such an important subject. □

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Expression of the genetic apparatus

John Scaife

Gene Expression. Vol. 3: Plasmids and Phages. By Benjamin Lewin. Pp.942/928. (Wiley: London and New York, 1977.) Hardback £24; \$40.65; paperback £10.25; \$19.50.

READERS of this journal will be familiar with the changing fortunes of microbial genetics in recent years. In the early to mid-1960s, its practitioners saw the genetic code unveiled and the birth of the operon. Five subsequent years of plenty were followed by a decline, as interest turned from lower to higher organisms and their development. Now the wheel has come full circle as the potential of bacteria and their viruses in genetic engineering is understood. As if to mark this development, the new volume in Benjamin Lewin's series on *Gene Expression* is devoted to plasmids and phages. These genetic elements have made a massive contribution to our understanding of the mechanism of heredity at the molecular level. They also happen to be suitable vectors of recombinant DNA made *in vitro*.

This series of books is intended to provide a critical analysis of the organisation and expression of the genetic apparatus at the molecular level. In this context, the present volume gives an excellent entry to the literature for advanced students of molecular genetics. It will also be a valuable reference work for final-year undergraduates. Rather than write a textbook, the author has manfully undertaken to review the whole field in some detail. The enterprise has been a profitable one. Indeed, his account of some systems is more incisive and digestible than the available specialist reviews. Readers will particularly appreciate the presentation of experimental protocols and models in diagram form, although grey rather than a brighter second colour can lead to ambiguity in some figures. Taken as a whole, the book with only a few important inaccuracies brings the general reader painlessly up to date in a fast-moving field.

Plasmids are now known to occur naturally in bacteria as widely different as *Staphylococcus* and *Agrobacterium*. The first of its kind to be discovered was the fertility factor F, which promotes mating between strains of *Escherichia coli* and, as it turns out,

other related enteric bacteria. It is a circular DNA molecule which, although much smaller (~2%) than the bacterial chromosome, is big enough to carry nearly 100 genes (94.5 kilobases). It can exist as an autonomous element but may insert into the chromosome.

Plasmids can vary in a number of respects. Some are unable to insert in the chromosome, others cannot mediate their own transfer by mating. They can carry genes of considerable importance in agriculture and medicine. For example, R factors are plasmids which confer multiple drug resistance on the host bacterium. Pathogens containing R factors have become a serious clinical problem, particularly since they can often transfer multiple drug resistance to other bacteria. Other plasmids determine the ability of some soil bacteria to utilise unusual materials such as camphor and toluene. At the same time, plasmids interest the molecular biologist as excellent tools to study, for instance, DNA replication *in vivo*, since they may be regarded and, more importantly, manipulated as dispensable analogues of the chromosome itself.

The bacteriophages form two large groups. The virulent phages are obliged to multiply on infecting their host. Most of them kill the host in the process. The important virulent phages of *E. coli* are discussed in this book, including the double-stranded DNA phages T4, T3 and T7, the single-stranded DNA phages λ X174, S13, f1 and M13, and the RNA phages f2 and QB. The temperate phages do not always kill the host. On infection they may enter the prophage state to give a lysogenic bacterium, in which the phage DNA is preserved as part of the host's hereditary material. There are temperate phages which are directly important to man, such as the corynebacteriophage β , which determines the toxin of diphtheria. Certainly the most studied of this group is phage λ , which is discussed in great detail in this work. Some readers will regret the omission of the phages of *Bacillus subtilis* and *Salmonella typhimurium* from this book. I was surprised to find the coliphage P1 given short shrift. P1 has the distinction of existing in the autonomous state as a prophage, qualifying for inclusion as both plasmid and phage. It is also an important vector of host DNA in genetic analysis by transduction, without which fine structure genetic mapping would be impossible.

The book devotes separate chapters to the different genetic elements, a profitable treatment for readers at the research level. A fuller index would probably have made it more acceptable to the general reader. The two opening

chapters describe the transfer material between bacteria, as naked DNA in transformation, and by mating (conjugation) between bacteria. These chapters provide a good account of the techniques of genetic mapping, and of the principles and mechanisms involved. They provide the background for later plasmid and phage chapters, and include discussion of the F factor. A separate chapter on plasmids focuses on R factors and col factors (which determine macromolecular antibiotics called colicins).

The molecular genetics of plasmids is examined in some detail. The mechanisms controlling plasmid copy number, transfer functions of F and related R factors, the molecular structure of plasmids as deduced from heteroduplex mapping techniques and the incompatibility between related plasmids in the same host are discussed in these early chapters. They also provide an informative account of studies on insertion sequences. These are specific DNA sequences, approximately 1,000 base pairs long, and are found in plasmids and at various sites on the *E. coli* chromosome. Interaction between insertion sequences can mediate insertion of F into the chromosome. Of perhaps even greater importance is the discovery that some R factor genes for drug resistance lie between insertion sequences and are transposed by them to new phages, plasmids and chromosomal sites. These movable genetic packages, now called transposons, provide a means by which drug resistance and other properties may reassort in nature.

The phage λ opts for lysogeny or growth shortly after infection. The molecular nature of this decision is not completely understood but it clearly depends on competition between mutually exclusive patterns of transcription. Of major importance in this competition are the factors facilitating or denying to the host RNA polymerase access to phage promoters. The disentanglement of these regulatory circuits by mutants, sequencing and other approaches makes a fascinating story. Dr Lewin's account of its development is one of the best currently in print. The virulent phages, T7 and T3, which are similar in size to λ , probably have simpler circuits. They code for an RNA polymerase of their own, and their growth is regulated at the level of transcription. The RNA phages, on the other hand, have important controls operating at the level of translation. The genetic organisation of RNA phages has largely been elucidated by direct sequencing, since these phages do not recombine.

The integration of λ prophage into the host chromosome provides a

beautiful example of site-specific recombination, which may serve as a model for the movement of transposons and analogous elements in higher organisms. The interaction uses enzymes which recognise different, specific, base sequences (recently elucidated by Landy and Ross, *Science*, 197, 1147; 1977) in the phage and host chromosomes, and promote crossovers between them. Most recombination, of course, is not limited to short sequences. Generalised recombination occurs between parental molecules of all phages but those with RNA as their genetic material. Models for the different types of recombination are discussed by the author in several chapters; and he gives a fair account of the current ideas in a changing field.

Most of the information encoded in a phage genome relates to the synthesis of the phage coat. More than 50 genes of phage T4 have this function. Notably, in phages T4 and λ , the assembly pathways have been mapped out by recording the stages reached in different maturation mutants. These

systems provide a way of studying spontaneous self-assembly and structural interactions between repeating subunits. They also lead to the conclusion that *in vivo* assembly is not spontaneous but is assisted by special accessory proteins. This is an area where Lewin's extensive diagrams and plates are most helpful.

Plasmids and Phages encompasses a wide range of modern techniques and ideas. They are described simply and well; and this presentation shows the dense logical and experimental substructure from which molecular genetics has grown. In addition, the author provides an excellent account of the early development of the major themes which continue to engage interest in the field. It is therefore fair to say that this book is valuable, not only as a research text but as a source which should be of considerable value to the future historian of molecular biology.

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Genetic engineering journalese

R. J. C. Harris

Biohazard. By Michael Rogers. Pp. 209. (Knopf: New York, 1977.) \$8.95.

THE title of the book does not alas describe it. What purported to be a description, in journalist's language, of the biohazard problems was a totally different book. The blurb of the cover outlines both the contents and the style: "Immediate, fascinating, frightening in its implications, *Biohazard* is the story of a current and major scientific controversy". The writer, Michael Rogers, portrays a "moral and scientific confrontation" reported "as it happened and where it happened".

Rogers outlines, and devotes most space to, the "history-in-the-making" meeting at Asilomar in early 1975 (pp51-100). He began, too, without an understanding of recombinant DNA; for him, then, the calculated breeding of plants and animals by selection and mutation was, in essence, genetic engineering. In chapters 2 and 3, we are led through the history of DNA (starting with Miescher and ending with Avery *et al.*) and acquainted with *Escherichia coli* and phages. The three chapters on Asilomar are preceded by

a recounting of the moratorium. Paul Berg enters the story with his dilemma about SV40 but fails to get the brief description accorded to most of the actors. Herbert Boyer, a soft-spoken San Francisco biochemist, discovered *EcoRI* which produced the "sticky ends" that allowed not only the original DNA strands to rejoin, but the coupling of the cut-ends of foreign DNA.

It is not clear how excerpts from the Asilomar meeting were reproduced with the apparently original comment of selected delegates. The taped official record was (p54) to be held for 50 years (*sic*). Did Rogers have his own tape, did he evade the 50 year rule, or had he merely adequate shorthand? In any event, this poses a general problem. Most scientists, even the most glib, prefer, if they are to be quoted verbatim, to see what they are purported to have said before it is published. Is it not courteous to ask the prior consent of the scientist before attributing to him remarks of any kind? These steps may have been taken for the US contingent where their scientists were usually quoted—for example, David Baltimore ("trimly bearded and clad in an embroidered Levi shirt") and Ray Curtiss ("imposing, long-haired") but not, probably fortunately, for most of the British. One who may recognise himself talks about K12 ("nice, quiet, boring person at least as far as his colon is concerned"). Two Britons at Asilomar are quoted (or misquoted) by name—E. S. Anderson from Colindale ("portly, imposing gentleman") and

Sydney Brenner ("bushy eyebrows, gleaming eyes, non-stop animation, mid-way between leprechaun and gnome"). One group, at least, was missing: those whose experience was in the realms of infectious diseases. Anderson drew careful attention to this (p63) and "glared coldly" as he did so.

Summing-up the meeting (at which some 10% were journalists, anyway) is well described. The problem is so acute—more akin to asking a committee to write a poem—that the most recent US meetings on recombinant DNA have compromised the organisers by drafting a statement (a letter in this case) and seeking adherents from among the meeting. Although Asilomar was attended by many microbiologists from different countries, only the British were singled out and the Russians are mentioned once.

The single chapter devoted to a discussion of biohazards begins with: "the only recorded civilian biohazard fatalities—the deaths of individuals who were in no way connected to a laboratory—occurred in London, in March 1973" and refers, of course, to the smallpox incident. Rogers uses this accident (albeit from a well-known human pathogen) to justify the widely felt concern about "new infectious organisms containing novel genetic material". Prominent among the concerned was, of course, Mayor A. E. Velluci of Cambridge, Massachusetts. The possibility (Velluci's "disease that can't be cured—even a monster") of a public, or ecological, disaster added bright new ammunition for the "town" in its confrontation with "gown". The author describes in some detail Building 41 at the National Institutes of Health at Maryland, which was built apparently for \$3.5 million, to handle the viruses which were expected by the US to emerge from the accelerated Cancer Program. The methods of handling biohazards, in genetic engineering research, might well have been stressed at greater length. In the UK, the Biological Safety Officers and their Safety Committees exist solely to assure the public (in the final shape of the Health and Safety Executive) that the containment level selected, whether this be physical or physical/biological, matches the risk and is thus safe.

The next chapter diverges from the story of succeeding conferences at which Rogers is becoming more confident, and more of a *confident*; he reverts to restriction enzymes, gel electrophoresis, complementary DNA sequences, molecular cloning and shotgunning. A crucial issue which is mentioned as a possibility is that of "libraries of cloned gene sequences" whether these be of prokaryotic (*E.*

coli) or eukaryotic origin (such as yeast, frog or *Drosophila*). The UK Genetic Manipulation Advisory Group (GMAG) is currently insisting that all unrecognised DNA fractions from shotgun experiments should be destroyed rather than preserved in collections, as is apparently the case for such libraries in other countries. This seems to underline the necessity of seeking international agreement, as indeed, ICSU, ESF, EMBO and WHO are trying to work towards on issues such as these. There can never be a case for seeking a general code of conduct for handling genetic recombination experiments, but the *principles* could well be agreed. Among these is the amassing

that might be discovered. For industry in the UK and even for academic workers, there is, however, a strong disinclination to declare plans and objectives to GMAG in the detail required. How this problem of confidential treatment will be resolved remains to be seen.

Sydney Brenner, who figured prominently in the Asilomar discussions—often apparently coming to the rescue when a particular meeting seemed to have been stalled—seizes an opportunity to advance the concept of a “disarmed bug”—biological containment to supplement physical containment which Paul Berg (p151) had condemned in a letter, as “overrated and,

they will even try to understand all the issues at stake. Will it be enough that the UK watchdog, the Health and Safety Executive, reinforces the advisory group (GMAG)? The public does, indeed, have four representatives on GMAG together with ASTMS, IPCS and TUC union members.

Public opinion may well be satisfied in the UK that laboratory manipulations are carried out safely in inspected premises (for the two higher containment categories). Are the US public so satisfied? No, they are not. According to Rogers, they do not trust the scientists, judging them to be mere self-servers, anxious to gain instant recognition and credit at the expense of the public and, moreover, encouraged and supported by the NIH which is largely composed of a peer group with identical motivation. Normally, the older generation is more conservative than the younger. This issue has converted the younger in the US into “nay-sayers”. The “yay-sayers” are their elders.

The scientific debate has elicited a response from the law-makers in the US and a counter-response from the scientists. It seemed, until recently, that a Federal law would be passed regulating the issues. The scientific community does, however, seem to be gaining the upper hand. If this opens the way for States to enact their own rules, Mayor Velluci for one will, no doubt, be satisfied. There will be some States who will be strict and some not so strict. In which will the experiments tend to get done? There is already a strong ground swell in the US in favour of relaxing the NIH's guidelines. After all, nothing has yet happened to create an ecological disaster, has it? the disarmed hosts/vectors that we currently have, seem to be more than adequate; Nature may even have tried many of the scientists' new tricks without the benefit of guidelines. Well, the pendulum was bound to begin the reverse swing somewhere, soon. I hope that a sensible mid-position can be found, and held.

This book sounds the occasional wrong note but it tells an interesting story of the US honestly and well, warts and all. An index would have been useful. The UK has only a passing reference (to GMAG) at the very end where the guidelines are damned with faint praise—“probably generated considerable scientific red tape”. Coloured, perhaps, by Rogers' rose-tinted spectacles? □

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Paul Berg (left) and David Baltimore (right)

Photos: NAS (Berg) and Noëleen Chedd (Baltimore)

of “libraries” from shotgun experiments, since, in the present state of genetic knowledge, the dangers of accumulating unknown, as well as known and recognisable, dangers, are not insignificant. Very well, these dangers are only potential, not actual. The public is unlikely to be impressed by such an argument. Wisdom after the event cannot be entertained.

The book contains an outline of many problems, but the solutions to very few, despite the author's recourse to the (recorded) wisdom of the experts. American industry, for whom the NIH guidelines are politically inhibitory, was particularly worried about two aspects; first, the maximum 10-litre scale proposed and, second, the necessity to declare to other research groups any safer host-vector system

while reassuring to the psyche, is hardly the line of defence . . .”. Since Berg lists “careless mouth pipetting” as a cause of breakdown of physical containment apparently in a very high category, the training and discipline of those who are to work in such laboratories obviously leaves an enormous gap to be filled. “The ideal safeguard”, wrote Berg (according to Rogers), “is ingeniously designed biological containment that can prevent escape and propagation—even with slobs doing the work on open benches”.

Yet another of the problems facing NIH's Advisory Committee on Recombinant DNA Molecules is simple enough to define. Scientists can hope to understand, and reason with each other; but where will the public stand? It is not to be expected that

Molecular politics for novices

Sydney Brenner

The Ultimate Experiment: Man-Made Evolution. By Nicholas Wade. Pp. 162. (Walker: New York, 1977.) \$8.95.

A VAST LITERATURE has accumulated in the few years since the issue of recombinant DNA research made its appearance. The documents in the case already fill several shelves, and there seems to be no end yet in sight. What began as a laboratory technique followed by some doubts expressed by scientists quickly generated a world-wide debate which spread over an enormous range of scientific and social questions. Molecular biologists were thrust into an arena very different from their laboratories and found themselves embroiled in discussions first with clinical microbiologists and safety engineers and then with lawyers, philosophers and politicians, trying to solve what in many cases were, and still are, insoluble problems. The debate was played out on the public stage, often with all the actors in full frontal nudity, so that what began as domestic drama soon took on, as Jim Watson put it, "the aspects of a black comedy".

Indeed, there were times when one came to believe that the real issue at stake was human rationality; and the new subject of "molecular politics" still demands a fine sense of the ludicrous.

Nicholas Wade has now written a book to help the ordinary reader through the tangled maze. He gives a simple account of the techniques used to join DNA molecules together, to introduce the beginner in molecular biology to the world of restriction enzymes, plasmids and shotgun experiments. Here too, one will find explained physical and biological containment. But more important than this technical background is that the novice in politics will get a clear idea of the development of the issue, from its beginnings at the Gordon Conference in 1973, when concerns about the research were first formally expressed, right up to the recent debate in the US on legislative means for regulating work on recombinant DNA. As readers of his articles in *Science* will know, Nicholas Wade attended most of the meetings that were held, and he is able to communicate something of the atmosphere of the debate, particularly as it has evolved in the US. There, as some of the foreign scientists who attended the Asilomar Conference discovered to their horror, the Press attend meetings organised by Federal agencies. At Asilomar, it became clear that their presence would ensure that not only the content but also the style of the argument would be relayed to the outside world. The sole public

objector to the Press at the meeting, when asked to explain his behaviour by an irate journalist, remarked that he "believed in the inalienable right of consenting adult scientists to make fools of themselves in private". That exchange with a man who represented a newspaper that had brought about the downfall of the President of the country together with the fact that two-thirds of the participants were American, led to the perception that the discussion of the issue would be strongly affected by the political and scientific conditions of the US. As the book clearly shows, time has proved this to be correct. The action is set firmly on the American stage and there are only passing references to what was going on in the UK and other countries. True, very little was happening and then only on a more subdued level; but there can be no doubt that the Ashby Report was an important element in helping the Asilomar Conference to decide what work should be done.

The book can only be comprehended in the context of American society. We must remember that the issue arose at a time when the Watergate episode had suspended trust in governmental institutions, when the radical university movement had lost its focus with the end of the Vietnam war, when the honeymoon between pure science and government had ended, and when, even in the US, resources had become limited. Only these crude facts and not abstract principles can explain such bizarre events as the alliance between the radicals in Cambridge, Massachusetts, and the Mayor of that town.

The reader will quickly find that the debate abounds with difficulties and paradoxes. Take the argument advocated by Sinsheimer and Chargaff that new artificial recombinants could generate an evolutionary catastrophe. This raises a deep scientific question which cannot be dismissed simply by Chargaff's reference to "the evolutionary wisdom of the past million years". We have to ask whether change in the biological world is limited by the extent of genetic variation or by selective pressure. It is this question which lies at the heart of the debate, and it cannot be avoided.

In our own time, an immense change has been wrought in the microbes associated with Man and animals. The enormous increase in antibiotic-resistant bacteria cannot be ascribed to the creation of novel recombinants out of nothing, but, as everybody knows, has been the direct outcome of the widespread and often indiscriminate use of antibiotics. The genetic elements conferring penicillin resistance must have existed before the discovery of penicillin; they were enriched because

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Photo: Andrew A. Stern for NAS

our technology produced an enormous increase in the concentration of this chemical in the world. Natural mechanisms of recombination ensured that these would spread, given the selective pressure exerted. Thus, it could be argued that mere existence is not enough, even survival is not enough; for any element to come to dominate the world there must also be a strong selective advantage and the means for its transmission. It is not widely realised that the Western region of the US is one of the world epicentres of plague, yet this is patently not a serious disease in that country. The reason is not that every plague bacillus in California is securely locked up in a P4 facility but simply that the environmental structure of a modern civilised society is incompatible with the selective propagation of that organism.

These and other issues are still with us, and the story is by no means ended. Yet, in looking back over the past three or four years something positive has been accomplished. The research work

has been allowed to continue, perhaps at a slower pace than many people hoped for; it is the results that are now beginning to emerge that will bring the greatest justification when the tumult has quieted and the dust has settled; and what will remain for the historians of the future will be the insights the technique will have given into the structure of genes in higher organisms. Whether or not any of the other benefits promised by some molecular biologists will have become realised is hard to say. When they appear, however, it will be society and not science that will be judged, because some of us will then want to know whether these benefits are to be used for the good of all humanity or merely to satisfy the selfish interests of some industrial societies. □

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Rational agriculture

J. G. W. Jones

The Famine Business. By C. Tudge. Pp. 141. (Faber and Faber: London; St Martin's: New York, 1977.) £3.95; \$8.95.

In writing about the future of world food supplies, authors are often tempted by one of two easy options: first, optimism that resources will be found to enable mankind to maintain the *status quo*, which in many respects is inequitable and dangerous for large groups of the World's people; or, second, total disaster which could only result in global famine and a massive reduction in population through starvation. Mr Tudge avoids both these extremes and charts a much more difficult course for food production.

He introduces his subject by assailing the myths of population, energy use, and aid to developing countries; there can be no repetition of the economic growth experienced by the Western World since the Industrial Revolution nor a solution to the problems of feeding the developing nations based on technologically advanced agriculture. On the one hand he rejects the idea of the "world farm", where each area of the world specialises in growing what it is best able to, and on the other the idea of national self-sufficiency. The solution he proposes is one of "rational

agriculture" which, considering the difficulties, he defines remarkably precisely as "one that makes best use of the land, while meeting the nation's nutritional needs and gastronomic aspirations." The physical organisation proposed consists of (a) regional specialisation to a degree for beans, cereals and potatoes, and (b) local self-sufficiency for fruit and vegetables together with pigs and poultry fed on plate wastes. Establishing a rational agriculture would be impossible in capitalist systems but what kind of political and economic framework would be best is not stated. Capitalism is excluded on the grounds of its need for continuously expanding economies which are not sustainable in relation to food supplies unless irrational buffers, such as meat production from cereals or food processing, are introduced.

In discussing the nutritional implications of rational agriculture, the author asserts that man has always been omnivorous; any imbalance towards carnivorousness or herbivorousness is due to the omnivorous man's capacity to adapt to circumstances, and especially to the commercial promotion of meat, since the Industrial Revolution. The protein myth is predictably debunked. A plea is made to use the peasant cuisines of the World which were based on austerity and evolved to make palatable and interesting dishes.

The food processing industry is attacked as being concerned with unnecessary operations designed "to impose industrial techniques on food production". Many of its claims are quite contrary to the facts; variety,

seasonality and localisation of food supplies are all lost. Is "convenience" really achieved when time and energy is spent on distant marketing at hypermarkets rather than in the kitchen cooking? Particularly adverse comment is levelled at what Tudge calls "ersatz" foods. The price of meat analogues should be compared with the price of the beans from which they are made rather than with the price of meat; it would then be seen that not only are they inefficient in the use of resources but also uneconomic from the consumer's point of view. The industrial production of single-celled proteins is technologically too advanced for the Third World, and when all is said and done is only suitable for animal feed and thus inefficient.

The book cannot be described as cranky. It is logical and engaging to read, and, except in his attacks on the food processing industry, the author is commendably restrained in his consideration of the implications of rational agriculture for technology and for capitalism. Politics, nutrition and commerce have been mixed with iconoclasm to produce a book which should appeal to the idealist in every reader.

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Curriculum change and social climate

Mary Waring

Science Textbook Controversies and the Politics of Equal Time. By D. Nelkin. Pp. xi+174. (MIT Press: London, Cambridge and New York, 1977). £9.10; \$12.95.

In 1969, the Californian State Board of Education's guidelines for school biology ruled that creation theory, as given in Genesis, be taught as a viable, scientific alternative to the theory of evolution, equal time being accorded to both. The target was, clearly, National Science Foundation-funded BSCS texts, with their evolutionary underpinning, and the ruling represented a victory for local creationist pressure groups. Attacks on evolution were nothing new, and biologists responded only slowly; eventually, however, legal and political strategies seemed to be restoring the situation. Then, a nationally-linked network of creationist activists turned their attention to a NSF-funded social science

course, *Man: A Course of Study* (MACOS), whose evolutionary underpinning was far more explicit in its use of animal behaviour to develop concepts about the nature of man, and which fostered ideas of cultural relativism. The course had already received wide acclaim and, by 1974, was being used in some 17,000 schools in 47 states. In 1975, however, after several local controversies, and following Congressional airing of the issue, funds for MACOS were terminated, sales plummeted, and a major re-evaluation of the NCF's (federal) funding of science curricula was instituted.

The causes and effects of action can never be explained in their own terms, but only in relation to all the events—and the network of interests, vested or otherwise—of which the action is part. Clearly, creationists were merely catalysing processes already latent in American society, and Professor Nelkin therefore sought for more widely-dispersed social and political tensions. Her interest had been aroused initially by the fact that creationists were representing themselves as *scientists*, a claim that enabled them, in attacking science textbooks, to circumvent dismissal on grounds of irrelevance or of infringement of academic freedom. Moreover, their focus on the highly sensitive area of the role of schools in value transmission enabled them to capitalise on a seemingly widespread concern over the erosion of traditional moral and religious values. Other significant tensions were concern for the control of hitherto 'unaccountable executive bureaucracies', for a questioning of scientific 'authority', and for lay participation in decision-making.

Professor Nelkin's account makes fascinating reading and her analysis is valuable in drawing attention to the significance of social climate in educational decision-making, but it is not entirely satisfactory. This is, perhaps, partly because boundaries have to be drawn somewhere (the area is a very complex one), but it is also because there is some woolliness in the discussion of, for instance, the nature of science and, in particular, the relationships between the sciences (especially when social sciences are included), and of the standing of evolutionary theory. The fact that MACOS is in fact intentionally directed towards the development of judgement and tolerance in a pluralistic, democratically orientated society is not made clear in her concern to juxtapose scientific and social values, and we get no explanation for social scientists' failure to defend their project. (Biologists do not seem to have regarded it as their concern.) Curiously, too, no mention is made of the possible influence of economic recession on both sales and funding.

Nevertheless, there is a mine of information and careful documentation here. The story is well told and is absorbingly interesting. The book is a most useful addition to the literature on curriculum change and social

climate and on pressure groups in science education. □

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Policy research perspectives

Stuart S. Blume

Science, Technology and Society: A Cross-Disciplinary Perspective. Edited by Ina Spiegel-Rösing and Derek de Solla Price. Pp. 607. (International Council for Science Policy Studies: London and Beverley Hills, California, 1977.) £20.

THIS volume offers a 'state of the art' review of that somewhat diffuse research field called here Science, Technology and Society (STS) studies, directed "mainly toward scholars in the various constituent sub-fields" (p3). Because the structure and value of the book can only be understood, and its strengths and weaknesses assessed, in the light of some appreciation of the structure of the field, I must begin with some general remarks on the study of scientific activity.

Research which the reviewer may choose to subsume under a heading such as STS in order to explore or (as intended here) to stimulate the 'intellectual integration' of the field is actually carried out from a variety of perspectives. Thus, sociology of science and the economics of research and development are both central, but most people working in these areas owe their principal allegiance to, and derive their research perspectives from, the well-established disciplines of sociology and economics. At the same time, there has emerged in the past 10–15 years a substantial multidisciplinary research activity, to which the existence of (for example) the Science Policy Research Unit at Sussex University, the Science Studies Unit at Edinburgh, and the Society for Social Studies of Science all bear witness. The fact is that the rapid growth, and institutionalisation, of both disciplinary and multidisciplinary research has not resulted in any real crystallisation of the field.

On the face of it, this apparently transitional intellectual structure has many parallels. Many new fields of scientific or technological research pass through phases in which some new integrative perspective competes, intellectually and professionally, with its parent disciplines. The early days of, for example, molecular biology, oceanography, radio astronomy, and materials science were like that. STS in fact differs from these in that 'the' new multidisciplinary perspective, which

ought to provide the basis for a new crystallisation, is itself fragmented. Broadly speaking, one nucleus has been science policy: the practical problems of allocating resources to science, organising research, stimulating and evaluating technological innovation, and so on. (Salomon's chapter in the volume under review shows how science policy research has been, in part, a response to the development and politicisation, of national science policies.) Equally broadly, a second nucleus has derived from the growing conviction among (some) historians, philosophers, and sociologists of science that the production of scientific knowledge has to be seen as having both epistemological and social dimensions which in fact interact. Thomas Kuhn's famous book *The Structure of Scientific Revolutions* was a major stimulus to this recognition of the need for cooperation.

What we have today, therefore, is a considerable variety of research approaches, and related problems and priorities, some of which (and these far from dominant) imply the need for greater cooperation between two or three relevant disciplines. For example, in addition to a perspective which relates sociologists, philosophers and historians of science, another begins to link economists and political scientists in the study of technological change.

In grouping their contributions into three sections, Ina Spiegel-Rösing and Derek Price have made a similar kind of categorisation of orientations to the field. The first section, seeking to be integrative, focuses mainly on the origins, institutional structure and cognitive structure of STS as 'a' subject. The second section looks at the achievements of social studies of science, taking the relevant disciplines *seriatim*, whereas the final one considers science policy-related studies, and is mainly written by political scientists. Because part 1 is closely bound up with the scope, utility, and future of the subject as a whole I will postpone discussion of it for the moment.

Part 2 contains chapters by a sociologist, two historians (of science and of technology), an economist, a psychologist, and a philosopher. One turns to these chapters with numerous hopes: of an outline of the various perspectives current in each discipline, their achievements, and their potentialities (especially for greater cooperation with other disciplines); and of some communication of the excitement of the research and of its currently crucial problems. Although some contributors (notably Fisch on

psychology of science) are greatly handicapped by lack of progress to report, on the whole one is not disappointed.

MacLeod's chapter on the social history of science is particularly successful. He shows that this remains a minority perspective within the history of science, because it rejects the common assumption that scientific developments can be understood in terms purely of their intellectual antecedents, without reference to the environment of discovery. Moreover, for the social historian (though not for the orthodox 'internalist'), this environment is itself a subject of interest, providing potential linkages with other disciplinary perspectives. One turns the final page of MacLeod's chapter both informed and enthused.

Freeman (on the economics of R&D) and Mulkay (on the sociology of science) provide the highly professional treatments one expected. Freeman outlines the perspective on science and technology found in each major school of economic thought, from Adam Smith, and indicates the findings of recent empirical work on technological change. He devotes considerable space to the kinds of question which might lead to greater cooperation between economists and other specialists (for example, whether government investment in industrially orientated R&D should supplement or complement—in terms of industrial sector and timescale of benefits—what firms themselves do), and ends with a plea for a "political economy" of R&D.

Mulkay concisely summarises the major achievements of the sociology of science since R. K. Merton's pioneering work of the 1930s. He might only be faulted for saying too little about the frontier points at which sociologists are coming to work with philosophers on the one hand (partly treated by Böhme) and political scientists on the other.

There is considerable overlap in part 3 between Sapolsky (on science, technology and military policy), Schroeder-Gudehus (on foreign policy) and Skolnikoff (on the international system), although each writer offers valuable insights. I did feel the need, however, for some (even implicit) notion of the uniqueness and coherence of science and technology; more sociological understanding might have been helpful. An adequate understanding of the changing relationship between scientists and political power (treated by Lakoff) must involve appreciation of the effects of this relationship on the ethical and normative system of science, the constraints on the occupants of advisory roles, and so on. These are (partly) sociological questions.

Still more important, though seemingly unrecognised, is the need for some kind of theory of technological change. For example, Sapolsky makes the interesting observation (p458) that the process of weapons development may have become

'autonomous': "so oriented to serving its own internal needs that it weakens security by generating weapons which are too costly to produce" in sufficient quantities. Layton, writing about the history of technology, refers (p127) to demands for more socially responsive technology as requiring "a change in the inner structure of technology". But what is this "inner structure" and how does it change?

In Nelkin's chapter, we turn from the disciplines of the social sciences to the real world problems posed for policymakers and citizens by modern science and technology. How can we make social assessments of technology? How can adversary (for example, court) proceedings cope with technical questions? When should we mount a giant "Manhattan Project" attack (for example, on cancer) and when should we more wisely invest principally in basic research? STS research has few answers here, and this has to be recognised in assessing the current usefulness of the field, and the book, to those who are concerned non-specialists.

I now return to part 1, which is most relevant to a general overview. It is certainly recognised here that the current state of STS research is not such as to throw much light on practical questions (other than in some very narrow areas). But part 1 should have gone further. These practical questions surely require,

as most practical questions do, an integration of insights of a multidisciplinary nature. It is clear from the book that in this field such integration, such crystallisation, has made little progress. And yet in many of the contributions one finds reference to problems and perspectives that promise or imply cooperation with other disciplines. The locus of these cooperative possibilities, which could have been traced out, might have served as a beacon to those wandering this terrain.

Some of those who work on STS will take pride in the achievements charted in this book. Others, among whom I include myself, are perturbed rather that so much has happened in so piecemeal, so incoherent a fashion. The vast creative effort which might have forged of this book a motor of real progress has not been made. Perhaps no-one could have done it, and the criticism is unfair. I can certainly say that anyone deriving sociological, economic, philosophical or policy research problems from science and technology should read the book. I cannot really recommend it more widely, and indeed I rather hope that my erstwhile science policy colleagues in Whitehall do not read it. It will not persuade them of the usefulness of this kind of work!

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Utopian dream

Marie Jahoda

Civilization in Crisis: Human Prospects in a Changing World. Pp. 303. By J. A. Camilleri. (Cambridge University: Cambridge, New York and London, 1977.) £7.50 (paperback £2.50).

CAMILLERI, a young Canadian political scientist at Le Tobe university, has performed a *tour de force* in this book. He aims at nothing less than a comprehensive diagnosis of the many factors which account for the "decadence of industrial culture", to establish a "utopian vision" and to propose a "realistic strategy for change".

The major part of the book is devoted to the diagnosis of the current crisis which Camilleri regards as a crisis threatening the survival of man as a species. His arguments go, however, in scope and spirit, beyond the usual doomsday forecasts, even though he uses ideas and assertions from that source where they fit his purpose.

The major symptom of the crisis is, according to Camilleri, widespread alienation: "... political apathy, social anomie and mental or emotional dis-

order are the product of the dual process of domination and alienation to which the technological society subjects the human psyche" (p13). Drawing on a wide range of literature, including the various social sciences, philosophy, cultural critiques and analyses of current economic, political and military events on a world-wide basis, he suggests that only a radical change in international and national institutions can avoid disaster.

In discussing the economic and political dilemmas of the world and their manifestations in increased inequality, underdevelopment and instability technology emerges as the major villain, leading to bureaucratic centralisation in capitalist and advanced communist societies, testing existing institutions beyond their limits and creating an intolerable imbalance. But on p222 Camilleri pulls himself and the reader sharply up in recognising that he is in danger of throwing the baby out with the bathwater: "... we may have created the erroneous impression that technology lies at the root of the modern human predicament. The problem of domination and dehumanisation that we have been describing cannot be attributed to technology as such, but only to the specific role assigned to it by the ideology of industrialisation." But he remains evasive on

how international inequalities can be reduced without some ideology of industrialisation in poor countries. There are various hints in the book suggesting that the Chinese experiment in social organisation may be one way out of the dilemma. But Camilleri is too concerned with the liberation of the individual to suggest a wholesale transfer of an experiment whose authoritarian character the Western world is beginning to realise as the perhaps inevitable counterpart of its remarkable virtues and achievements.

Camilleri's "utopian vision" is culled from a number of thinkers of most diverse outlook—Marcuse and Pope Paul VI, Marx and Teilhard de Chardin, Mao and Gestalt therapists, Illitch and (surprisingly and only in a note) Skinner. The author is right, of course, in saying that any critique of the present involves at least logically a concept of what could be, and equally right in not proposing a blueprint but rather a set of human values which should be maximised in a new cultural synthesis. Central to this deliberately vague utopia is the small organic community where "the liberation of human sensitivity and sensibility is made a primary goal of social interaction" (p187).

It is to the author's credit that he does not shirk the question of how to make this often dreamt dream come true, even if his answer is more appealing for its sincerity and morality than convincing as a solution. Camilleri advocates a non-violent cultural revolution which he sees already emerging in the rich world: "Once the counter-institutions, whether it be free universities, underground churches, free presses, community schools, worker-directed enterprises or neighbourhood-controlled welfare services, have demonstrated their efficiency and legitimacy through a continuous process of innovation and experimentation based on service and direct participation, the established institutions are likely to collapse with little or no coercion required..." (p254).

One can hardly blame the author for not having solved all the world's problems. Nobody else has done so either. But such naiveté, together with many unexamined assertions and some inconsistencies in the book, overshadow its positive features.

The value of the book to a reader wishing to appraise the evidence on which the author bases his arguments would have been enhanced, had the index included references to the Notes.

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Hard and soft paths

P. F. Chapman

Soft Energy Paths: Towards a Durable Peace. By Amory B. Lovins. Pp. 254. (Ballinger: New York; Wiley: Chichester, UK, 1977.) Hardback \$15.40; £9 Pp. xx+231. (Penguin Books: Harmondsworth, UK, 1977.) Paperback 95 pence.

AN energy policy involves a projection of energy demand at some future date coupled to a means of supplying the requisite quantities of energy in appropriate forms. Although there are radically different policies advocated, each with its own mix of supply technologies and demand estimates, virtually all policy analysts are agreed on the ground rules that must be used to evaluate or judge a policy. The first rule is that the demand projection and methods of energy supply should neither require nor impose any substantive change in consumers' lifestyle. The second is that all the technologies involved, either in supply or utilisation, should either be proven or have a demonstrably high chance of feasibility. The third rule is that the policy should provide energy at least cost. If this latter cannot be demonstrated then it is essential to demonstrate that all investments show an adequate return on capital. The differences between advocates of different policies can, under these agreed rules, be boiled down to different choices of important parameters, with each author accusing others of making choices of convenience. Since many of the important parameters are in principle unknowable, this leaves plenty of room for endless, and fruitless, debate.

In his new book, *Soft Energy Paths*, Amory Lovins attempts to use the agreed rule book to show that the "hard technologies" advocated by the establishment are significantly less desirable than the "soft technologies" proposed by environmentalists, conservationists, lovers of peace, and... Amory Lovins. The basic thesis of the book was published by Lovins in *Foreign Affairs*, an influential US quarterly. The article is reproduced as chapter 2 of the book, the remaining chapters serving to embellish and document the arguments. Although the approach and data in the book are clearly aimed at influencing US energy policy, Lovins makes it clear that the same principles and analyses could, and should, be applied in all industrial nations.

The 'hard' path described in the book is based on the policies of a number of US agencies, including the Energy Research and Development Agency. This policy has energy demand (in primary energy terms) doubling by the end of the century and increasing by as much again by 2025. Over the 50-yr period, oil and gas decline from a 75% market share to about 10% with coal and nuclear power accounting for both the growth and the substitution. Lovins argues against this policy in terms of changes in lifestyle and unacceptably high costs. He shows that the massive use of technical resources, the potentially enormous environmental damage and the security measures required for a massive nuclear programme, will alter the way of life of most Americans. He also argues, with considerable conviction, that the development of nuclear power will lead to the widespread proliferation of nuclear weapons with potentially enormous effects on 'lifestyles'.

Lovins' critique of the hard path on cost grounds is similar to many previous analyses. There is no doubt that the costs of nuclear systems are becoming embarrassingly large, and there seems to be no decrease in their escalation at a rate substantially faster than the rate of inflation. To this embarrassingly high cost, Lovins adds the equally enormous costs of distributing electricity to consumers. He then makes the numbers absurdly high by dividing by an unrealistically low 'load-factor'. This is silly, since it provides ammunition for those who would knock down an otherwise sound and important argument.

Underneath the critique of the hard path, there are a number of principles which show Lovins' previous training in physics. The first is an obvious distaste for waste of a physical commodity which, by any route, is going to be more expensive in the future. The second is the mismatch implied between the high quality of energy supplied to consumers (electricity) and their low quality energy requirements (low temperature heat). Lovins attacks the question of mismatch, directly as a matter of principle, without drawing on the obvious economic argument that rising prices will push consumers towards more appropriate fuels. This is perhaps because Lovins subscribes to Galbraith's thesis which puts sovereignty in the hands of producers not consumers.

Improving the match between energy supply and end-use function is one of the major starting points for the 'soft' path synthesis. Another is that if one can use ambient energy sources, particularly wind and solar, then one

can avoid most of the costs of energy transmission. To these significant advantages, Lovins then adds a number of very dubious cost advantages associated with small scale mass production (as opposed to large scale one-offs). It is here that Lovins falls into the ubiquitous trap of choosing data that happily proves his case. Two examples illustrate the type of problems involved.

First, Lovins suggests that car engines provide a system of electricity generation at a cost of \$40 per kW compared with power station costs of more than \$400 per kW. Lovins omits, however, to cost the 100 kW generator that needs to be coupled to the car engine. More seriously, his argument for mass production is undermined when it is realised that a car engine is worn out after 4,000 h of running time, whereas a large generator is expected to run for 6,000 h per yr for 20 yr or more.

The second example concerns the cost of solar energy systems. Lovins is only concerned with the cost of active solar systems, employing flat plate collectors and large hot-water heat stores. He arrives at a figure of \$100/m² of collector *plus* a m³ of storage. In the UK, an optimistic cost for solar collectors is £50/m². The cost of storage in the UK has been put at £300/m³ by the Buildings Research Establishment. By using cheap agricultural tanks and the cheapest methods of installation and insulation this cost can be reduced to £40/m³ for moderate tank sizes (over 40 m³). Thus UK costs are at best £90 per (m²+m³), equivalent to \$150. This estimate is, however, still too low, since it ignores the cost of loss of house space and control equipment. The 50% difference between the most optimistic UK figure and Lovins' data is sufficient to cast serious doubts on the cost-effectiveness of active solar systems.

There are other serious criticism of the proposed 'soft path'. Throughout the book Lovins fails to produce comprehensive data on how much energy is to be supplied by what source for use by which consumers. Many attractive back-of-the-envelope calculations fall down when examined in detail, especially in complex energy systems. There is also no adequate discussion of the environmental effects of the soft path. In this respect, windmills are clearly cast in doubt, and outside the US there are few countries who could afford much acreage for growing 'biomass' fuels.

Although Lovins' book does fail on a number of technical issues, this is more than compensated by his breadth of approach. Energy policy questions are cast in a very general sociopolitical framework and a number of important questions raised. Of these, the relation-

ship between nuclear power, centralised control and weapons proliferation are the most important and urgent. Is any energy policy important enough to threaten every nation's foreign policy?

Although Lovins clearly identifies many of the central issues, one is left with a number of important questions unanswered. The last four years have seen some remarkable shifts in attitudes towards the alternative (or soft) energy technologies with most nations now actively supporting a very diverse energy research and development programme. There remains, however, an enormous commitment to nuclear power. The costs are embarrassing both the utilities in the US and the Central Electricity Generating Board (CEGB) in the UK. (Here, it is interesting to note that the CEGB is supporting British Nuclear Fuels Limited at Windscale, despite their admission that reprocessing will cost them about £300 million more than obtaining fuel from uranium ore).

Energy forecasting

Walt Patterson

Energy or Extinction? The Case for Nuclear Energy. By Fred Hoyle. Pp. vii+81. (Heinemann Educational: London, 1977.) Paperback £1.50.

COSMOLOGY does not, on the face of it, have much to do with the subtleties of terrestrial economics and politics. Nevertheless, Sir Fred Hoyle has taken a flying leap out of the galactic depths and into the middle of one of today's most immediate and heated controversies. Sir Fred, of course, is no stranger to controversy. He is, however, something of a stranger to energy policy, as he demonstrates repeatedly in his lively polemic *Energy or Extinction?* He writes, as usual, with flair and enthusiasm. All too often, however, he finds himself out of his depth.

The tone is set by Sir Alan Cottrell's Foreword to the book: "It is about energy: about the alarming prospect that oil will soon run out and not be replaced by anything else. It shows that—contrary to an influential belief—we do *not* have time, that there is *no* practical alternative to nuclear energy . . ." After this outburst, Sir Alan goes on to deplore the "hysteria of the anti-nuclear environmentalists". Both the Foreword and the book it introduces bear witness that environmentalists have no monopoly on hysteria. Sir Alan's allusion to the book's "refreshing commonsense" runs afoul of its

Wherein lies the source of this commitment? Is it the massive investment in nuclear technology? Is it the enormous institutions built-up on nuclear technology employing so many highly qualified scientists and technologists? Or is it perhaps the military link? Lovins book is causing ripples in the US and may encourage President Carter to go further with his non-proliferation policies. It will certainly provoke interest and controversy in the UK. But before any action can be taken on a 'Soft Energy Path; Toward a Durable Peace', someone will need to analyse the soft path in far greater detail *and* be able to account for the commitment to the hard path policies presently being followed by all the industrial nations of the world. □

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very title; do we really have to choose now between "energy or extinction?" It is an emotional catchphrase that begs almost all the important questions.

Sir Fred's approach to energy policy is based on a series of non-negotiable assertions couched in stark all-or-nothing terms: "There can be no disagreement with the statement that world reserves of coal, oil and gas can provide an adequate energy source for only a limited future . . . nor can it be contested that most of the world's population . . . will die in a disastrous catastrophe should an adequate energy source not have been developed by the time that reserves of coal, oil and gas become exhausted . . . nor can there be any serious debate over the statement that the only alternative energy source *presently known to be technically viable* (italics in original) is energy from the nuclear fission of uranium or thorium". If Sir Fred genuinely believes that none of these assertions can be disputed, he has somehow failed to notice the boiling ferment now engulfing energy policy worldwide.

He has also overlooked a fundamental consideration which is likewise absent at key places elsewhere in the book: the matter of cost. Even "reserves" of fuel depend critically on the extraction cost assumed, as well as the value assigned to the fuel in use. "Most of the world's population", however, presently depends largely on forms of energy which are not bought and sold. Technical "viability"—surely a contradiction in terms—is long since proven for many energy technologies, and is not the central problem. The primary problems are economic—how much money and time we are prepared to

spend on various aspects, especially investment; social—the costs and benefits, and how they are to be apportioned, especially employment; and political—who shall decide, and how. Despite Sir Fred's suspicions, the factor now impeding nuclear development is not the third but the first of these: the cost and the lead-time.

Sir Fred belongs to the traditional school of energy forecasters, whose motto is "Think of a number and double it". He notes that Britain's per capita annual use of energy is only about half that of the US: "this of course is the over-riding reason why the standard of living is lower in Britain than it is in America. By doubling the British energy flow, our standard of living would rise inevitably towards the American level". That being so, why do we not simply turn up the central heating and open all the windows? The putative link between GNP and energy use looks more tenuous every year, to say nothing of that between GNP and 'standard of living' in meaningful terms. Sir Fred, however, desires "to raise the standard of living of everybody in the world to the American level"—presumably by American cultural standards, universally acknowledged as the acme of human accomplishment. It seems not to trouble him that the immediate requirement of most of the planet's people is for local energy systems, using local skills and most of the planet's people is for local money. In most Third World countries, capital-intensive centralised electricity systems are a comprehensively inappropriate misallocation of effort, as even the World Bank is coming to accept.

Sir Fred is convinced, however, that this is how we must deploy our resources. He is concerned with energy supplies 30,000 years hence, but has a shakier grasp of more relevant time-scales. "It is a mistake to think that conservation could go very far towards mitigating our future need for energy. Conservation could moderate the need, but by no great margin, for *if the margin were great, conservation would have happened already*". Our present pointlessly wasteful infrastructure—buildings, transport, industrial plant—was established during a period of absurdly cheap energy. This infrastructure cannot be replaced overnight with one more appropriate to a time of high energy price. Such replacement, however, is already underway; and it does not in any way imply—in Sir Fred's phrase—a "hairshirt economy" (see, for instance, the evidence given by Friends of the Earth to the Windscale inquiry).

Sir Fred's lack of first-hand acquaintance with the work and views of Friends of the Earth led him and his

publisher into a slight contretemps. The opening chapter of the book suggests in lurid terms that critics of nuclear power like Friends of the Earth are agents or dupes of the Kremlin. Before the book's publication, however, Sir Fred and Heinemann added and circulated a statement, withdrawing all imputations of 'reds under the reactors'; and two staff members of Friends of the Earth were guests at the book's publication party.

There are a few lapses which Sir Fred should have caught: "molecules" of salt? His discussion of the nuclear option itself suggests that he needs further homework here as well: "fuel elements" or "fuel assemblies", but not "fuel cells", and power reactor control rods are not made of cadmium. His discourse on radioactive waste commences with another brisk wave of the hand: "I have always been asked about the 'problem' of the disposal of radioactive wastes. There is no problem, and the thought that there is, if the concern is genuine, can only come from ignorance and from a fear of the unknown". Perhaps he should have a word with Sir Brian Flowers, whose ignorance and fear of the unknown are conspicuous by their absence. Sir Fred's otherwise generally lucid prose ties

itself in knots during his attempts to banish the non-problem. He also gets the half-lives of both strontium 90 and caesium 137 wrong.

Much the most unsatisfactory paragraph in the book, however, is his extraordinary dismissal of the issue of nuclear weapons proliferation. Rarely have I seen such wilful sophistry as the assertion that linking nuclear energy and nuclear bombs is on a par with linking "eating a piece of chocolate and exploding a hand grenade" because "both (are) manifestations of chemical energy". This will not do, and Sir Fred should know better.

It is ironic that—assuming we move quickly to resolve the nuclear weapons problem—the so-called "anti-nuclear environmentalists" are today the optimists, who foresee an abundance of options and are working eagerly to respond to the challenge. Those who insist that the choice for humanity now lies between nuclear energy and freezing in the dark are—in Sir Fred's verbal boomeranging—"scaremongers whose prime concern is to frighten people". Cheer up, Sir Fred—from where we sit the view is really rather encouraging. □

Walt Patterson is Energy Consultant to Friends of the Earth (London) Ltd.

Epic work on climate

John Gribbin

Climate: Present, Past and Future. Vol. 2: Climatic History and the Future. By H. H. Lamb. Pp. 835. (Methuen: London; Barnes and Noble: New York, 1977.) £38; \$85.

THE arrival of the second volume of Professor Lamb's epic on climate has been eagerly awaited by everyone working on studies of the climate. The first duty of any reviewer is to report that the book is no disappointment, fulfilling the high expectations of those who have already found the first volume indispensable. That said, it might seem that further comment is superfluous; but since 1972, when volume 1 of *Climate: Present, Past and Future* appeared, debate about climatic change has moved so much into the realms of the planners, politicians and concerned laymen with no specialist training in the subject, that the book is now of far wider significance, and likely to have a much broader impact, than even the author could have imagined when he began his mammoth task twelve years ago.

Recent vagaries of the Earth's atmospheric system have been front-page news around the world, and include record-breaking drought followed by record-breaking precipitation in England; simultaneous severe drought in the western US and heavy snowfall in the east; failures of the monsoon in some parts of the world; and frosts in Brazil, which have been used as an explanation of recent dramatic increases in the price of coffee. The latest annual report of the UK Meteorological Office was the subject of a first leader in *The Times* in which the writer commented "there seems little doubt that the world's climate has been changing", and was taken to task by a member of the School of Geography in Oxford who argued that it has all happened before and refused to accept "that recent weather events in Britain provide reliable evidence for a significant climatic change".

When the world's weather seems to be turned topsy-turvy, but specialists can be found prepared to argue any of the cases that: (1) nothing unusual is happening; (2) we are hastening towards a new Ice Age; or (3) present troubles are caused by the greenhouse effect of human activities which produce carbon dioxide and warm the globe, the wretched non-specialist—

who, in this case, may be involved in attempts to improve agricultural productivity to meet the needs of a growing global population—needs some secure foundation on which to base his interpretation of climatic events. Professor Lamb's books provide that secure foundation, and the second volume is of particular importance to anyone concerned in an empirical way with changes in climate, concentrating as it does on climatic history and on implications for the future.

The simplest and most reliable guide to the climate is that if something has happened before it can happen again. Professor Lamb's look at what has happened before covers successively, and in increasing detail, the long history of the Earth, the Quaternary, postglacial times, the historical record, and the period of instrumental records. This natural division of attention reflects, of course, the amount of detailed information available, but also concentrates on the periods of most interest now—the most recent fluctuations in climate. With an outline of more distant, long term events as background, the changes since the most recent full glaciation provide the appropriate insight into what may be happening to climate today. Suggestions that man's activities are solely responsible for increased Saharan desertification, for example, should be considered in the light of evidence about past development of the desert; this would seem to indicate that both the Sahara and Kalahari deserts spread towards the equator during periods of cooling climate.

Cooling phases are likely to be of particular interest at the present time, since the consensus of various forecasts assembled by Professor Lamb is that we are likely to see a continued global cooling over the next 30 years or so. This is not to say that we yet have reliable means to forecast such changes in detail, but "encourages belief in the possibility of developing a real forecasting skill in relation to such periods ahead, and suggests that some useful guidance has already been obtained from this scientific research". The joker in the pack, however, is the prospect that mankind's activities, most likely the carbon dioxide greenhouse effect, will introduce a significant new variable into the climatic system just at a time when we are on the brink of understanding the natural processes.

Professor Lamb's great achievement is that he has assembled in one place a vast store of information about the climate, encapsulating present knowledge at this critical watershed in the development of such an understanding. As a sourcebook for everyone working on the subject, the book would be

invaluable even if it were not well written; in fact, the author presents his material (from the implications of plate tectonic theory to the effects of mankind's activities on climate) so clearly that the volume would be a pleasure even to the casual reader who might dip into the sections on historical changes in climate and effects on human activities. Alas, I use the word "might" advisedly; for the price of the present volume is so great as to preclude any casual reader, along with many who have a serious interest in the subject.

At £38 (in the UK) for a single volume—even one of major importance, well written and more than 800 pages long—the publishers must surely be reaching the point of diminishing returns. Can any working scientist possibly buy such a book? And, indeed, can all of the libraries that might wish to have it on their shelves justify the cost? It must also be mentioned, in view of the large capital investment such a book represents, that although the volume's presentation and layout is, overall, of a high standard many of the figures have not been re-drawn into a standard format, but are simply reproduced from their original sources. Although all are acceptably clear and no harm is done to the intelligibility of the book (except where the labelling of the figures is retained in its original Norwegian), this is hardly the kind of penny-pinching usually associated with such an investment. The presentation and cost of the two volumes may have seemed appropriate when the project was initiated, but in view of the much more widespread interest in the topic now it would be good to see the whole

work available in more accessible format—perhaps as a series of three or four paperbacks which might be purchased individually as finances permitted.

But for anyone fortunate enough to possess the book and its companion volume the intrinsic value is unquestionable. It is impossible to do justice to Professor Lamb's achievement in the short space available here, and it will take months to begin to absorb the impact of this appraisal of the background to present-day climatic problems—followed, I am sure, by a lifetime in which it will remain a much used and valued reference. Perhaps the final words should be left to the author. First, a statement of intent from the Introduction which aptly describes the work: "This book is an attempt to marshal existing knowledge of climate and its behaviour, so as to provide a basis on which forecasting techniques will have to be developed".

And, finally, from a footnote (the many comprehensive footnotes are themselves one of the major delights of the book) commenting on those historians who do not accept that climatic change was contributory to the cultural decline and other disturbances of the Middle Ages in Europe, a remark which is strikingly apposite in view of the current climatic debate: "There is great need for open minds and a careful integration of knowledge". □

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Encyclopaedic jungle

Peter J. Smith

Planet Earth: An Encyclopaedia of Geology. Edited by Anthony Hallam. Pp. 319. (Elsevier/Phaidon: Oxford, 1977.) £7.95.

THE publishing world seems to have gone mad as far as the production of geological dictionaries, encyclopaedias and the like is concerned. I reviewed three such volumes earlier this year (*Nature*, 266, 101; 1977); several were then already in existence; another appeared a month or so ago; I have a new one to review here; and the publication of at least two more is imminent. This field, once so barren, is rapidly developing into a jungle; so this seems a good time to hack a way

through the undergrowth before it rises even higher.

Of course, not all works of the genre under consideration here are aimed at the same audience and not all cover precisely the same ground in precisely the same way. The massive *Glossary of Geology* (American Geological Institute: Washington DC, 1972) covers the whole range of the Earth sciences with some 33,000 entries which are generally short and highly technical. This is the working Earth scientist's bible and is likely to remain so, at least until the Institute summons up the courage to produce a new (third) edition. Not to be dismissed lightly, however, is the more humble *Penguin Dictionary of Geology* (D. G. A. Whitten and J. R. V. Brooks; Penguin: Harmondsworth, UK, 1972) which contains far fewer terms than the *Glossary* but devotes rather more words to each. Its language is perhaps best described as semi-technical, which makes the book useful to specialists

and comprehensible to students but hardly the sort of work the general reader would care to browse through.

The most serious objection to the Penguin dictionary is its weakness on geophysics, a gap that is filled to some extent by *A Dictionary of Earth Sciences* (S. E. Stieglar; Macmillan: London and Basingstoke, 1976). I say "to some extent" because although the gap is undoubtedly filled, the relative shortness of the entries in the Stieglar volume is not very helpful to those with no previous knowledge of the subject. This is a trap generally avoided by *Earth Resources: A Dictionary of Terms and Concepts* (D. Dinley et al.; Arrow: London, 1976) with its longer, more informative entries, although, as its title suggests, this book is narrower in scope.

If a distinction is to be made between dictionaries and encyclopaedias, all the books mentioned above, whatever their intended audience, would best be described as dictionaries; their entries, though generally not single-sentence definitions, are short and arranged alphabetically; and illustration, if any, is limited to a few line diagrams. By contrast, *The Planet We Live On: Illustrated Encyclopedia of the Earth Sciences* (C. S. Hurlbut; Abrams: New York; New English Library: London, 1976) comes closer to the popular idea of an encyclopaedia in that it contains hundreds of diagrams and photographs as well as numerous colour plates. Unfortunately, as I have had cause to remark before, this book contains so many inaccuracies that the whole stock is best left to rot in the publisher's warehouse. The point of mentioning it again here, however, is, first, to reiterate that warning and, second, to report that it is now possible to recommend to the general reader a book which, though similar in aim, is far superior in execution.

At the risk of being accused of using unscientific terminology, I can only describe *Planet Earth: An Encyclopedia of Geology*, edited by A. Hallam, as beautiful. It is nice to look at; colour photographs and/or diagrams adorn almost every page without making for simply a picture book with incidental text. It is accurate and authoritative; almost all the writers have been chosen from the ranks of those still active in the subject "because an understanding of the Earth depends on an appreciation of the fruits of recent research". It is for the most part well written—no mean achievement given the literary standards of a great many research workers. And it has a remarkably wide coverage, including some topics, such as engineering geology and the history of geology, which are all too often ignored.

It is also unconventional, in that it is arranged not alphabetically but in nine major sections each of which is subdivided into a series of closely related subjects. Thus, to take but one example, the section, "Processes That Shape the Earth", includes discussion of continental drift, weathering, earthquakes, diagenesis, and so on. The effect of this arrangement is to give the book a remarkable coherence. The pleasure of reading a text broken up into a series of 'terms' related only by the chance occurrence of their initial letters is strictly limited in my view, however useful such a book may be for reference. The delight of Hallam's approach is that it has produced not only a good work of reference (for the book has an excellent index) but also a more or less continuous text which cleverly directs the eye towards horizons beyond that originally sought. In short, it encourages the sort of pleasurable self-education that the disjointedness of an alphabetical arrangement can hardly begin to provide.

Provocative but not profound

David Davies

Ten Faces of the Universe. By Fred Hoyle. Pp. 224. (Heinemann Educational: London, 1977.) £4.80.

This is the fourth book, to my knowledge, out of Cockley Moor this year and its publishers call it "perhaps Fred Hoyle's most provocative and profound book yet". Provocative it certainly is in parts—small parts—but profound it is not, and the lack of profundity only serves to devalue the provocation to something rather trivial.

The ten views of the universe are respectively God's, the physicist's, the mathematician's, the astrophysicist's, the expanding, the origin of the, nobody's (quantum mechanics), the geophysicist's, the biologist's and Everyman's. Hoyle has devoted an average of twelve pages of text to each; the rest of the book is made up of ten pages of chapter headings, ten more of pictures of people central to each chapter (with the relevance frequently left to the reader to work out); forty-seven pages of illustrations, many of them barely relevant; eight pages of star maps and coordinates; three pages listing details of all the elements; and four pages completely empty. The intended audience is American, to judge from the spelling and the baseball analogues,

So Hallam has nothing to fear from Hurlbut. He does, however, have more serious competition in the form of *The Physical Earth* (Mitchell Beazley: London, £12.50), one of the volumes in the much publicised Mitchell Beazley *Joy of Knowledge Library*. Despite the title, about one-half of this book is devoted to plants, animals and agriculture, although to the general reader the Earth science section will appear as attractive as *Planet Earth*, and for similar reasons. If forced to choose between the two books, I would go for Hallam, partly because the space devoted to the Earth sciences proper is greater and partly because the rather higher proportion of text to illustration somehow imparts to Hallam's work less of a coffee-table feeling. Besides, there is a considerable price differential. In comparison with books of similar format and physical quality the cost of *Planet Earth* is remarkably low.

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and presumably at the intelligent layman level.

The book starts on a poor note. Hoyle gives the impression he will discuss the place of a God in or outside the Universe. But he soon gets distracted into rambling anecdotes about his solution for Northern Ireland, how children learn mathematics, what his schooldays were like, and how Galois met his death. Thereafter the book is not free of its irritating diversions, but does follow a more orderly path. Hoyle certainly would not claim to be presenting other than thumbnail sketches of physics, mathematics and cosmology but there are some nice things therein, and insights that even a hardened scientist might find useful. The two chapters on geophysics and biology, not so close to Hoyle's lifelong interests, are accordingly more pedestrian.

Then we come to Everyman's Universe which is about energy, population and the collapse of society. Hoyle suggests that he could have devoted a whole book to these subjects. It is certain that a chapter tacked on the end is totally inadequate and fails to give much impression of deep and sustained thinking. Hoyle, like many others, is worried about population growth, but unlike most others he tries to discuss it in terms of simple-minded graphs and immensely accurate prediction. The world will collapse into a Dark Age in 2025, give or take a decade or two. How is this figure reached? We are not told. Why should productivity (whatever that means) and population collapse so dramatically? Because the Roman Empire did. Why should the

phenomenon "perhaps have a cycle time of about 500 years"? We are not told.

Hoyle the scientist has become Hoyle the crystal-ball gazer but retaining a pseudo-scientific veneer of graphs, cycles and so on. And his draconian proposal that we should stop all this growth by heavily taxing three-children

families just comes as a flashy bit of provocativeness which really advances serious thinking on problems in the real world very little.

In all, a strangely mixed book, at times charming, at times half-baked. □

David Davies is Editor of Nature.

Cosmic gushers

Roman Znajek

White Holes: The Beginning and End of Space. By John Gribbin. Pp. 200. (Paladin: London, 1977.) £1.50; \$4.50.

A WHITE HOLE is like a black hole, but with time running backwards. Nothing can get out of a black hole, and nothing can get into a white hole. Whenever a black hole is formed, matter that is unable to withstand its own gravitational field collapses to form a point called a singularity. A white hole is *caused* by its singularity. The latter is best thought of as a remnant of the initial singularity from which the Universe began in the Big Bang. According to John Gribbin, if we are prepared to accept the existence of black holes then "the rest of the new astronomy inexorably follows", and that includes white holes. His book is intended to be a popular account of some of this new astronomy.

Now nobody knows for certain what a white hole singularity is going to do, and indeed nobody knows why it should be there at all. It is, however, not unreasonable to suppose that at some stage in its career it will start spewing things out and turn into a cosmic gusher, as Gribbins like to put it. Because the amount and nature of the gush is completely unknown, Gribbin has no difficulty in 'explaining' various highly energetic phenomena of extragalactic astronomy in terms of these cosmic gushers.

The conventional view is that quasars, radio-galaxies, and so on, derive their energy from matter in strong gravitational fields. For example, when gas spirals in towards a black hole very large quantities of heat are released through friction. There are reasons for expecting that kind of process to occur, even though there is much debate over the details. There are no reasons for expecting white holes to occur. Gribbin disagrees. According to him, the Big Bang is a white hole, and so there is every likelihood of there being others. But the universal Big Bang is a very different

phenomenon from the local white holes he thinks are responsible for quasars. What Gribbin cannot explain is why the Universe's initial singularity should have been deformed in such a way as to give rise to white holes.

Even if white holes did exist in the early Universe, they would have been very rapidly destroyed. In 1974, Douglas Eardley showed that white holes are unstable. A white hole attracts radiation and matter. But this material cannot get into it. It piles up on the surface of the hole, getting continuously accelerated. An exponentially strengthening 'blue sheet' is formed, which twists round the gravitational field and turns the white hole into a black hole. The black hole remains, because black holes are stable. The process is very rapid, and only ridiculously large white holes would survive into the present epoch.

It is interesting to see how Gribbin tackles this problem. First of all, he refers to Eardley's work as a "suggestion". He then says that "better physics is needed to deal with the situation". This is correct in so far as we don't know why the white hole is there, but given its existence Eardley's argument is immediately applicable. Gribbin finishes by claiming that this difficulty "is more than compensated for by the amazing discovery in the mid-1970s that black holes can explode". This is the cue for five pages on the Hawking process, which is interesting in itself but completely irrelevant to Gribbin's cosmic gushers.

The possession of unconventional ideas does not disqualify a person from writing a popular book. But he should at least give a good account of the orthodox view. Gribbin doesn't. The saddest thing about his book is that his astronomy is old. It is five years or so out of date. In the sixties astronomers were often baffled by the objects they saw through their telescopes, and were prepared to believe in all kinds of curious explanations. It now seems that there is no need to invoke unlikely objects such as white holes in order to understand quasars. White holes are old-fashioned. □

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Amplifying by stimulated emission

Harold Weaver

Celestial Masers. By A. H. Cook. Pp. 135 (Cambridge University: New York, London and Cambridge, 1977.) £7.50.

ANY new technological development that makes possible astronomical observations in a previously unexplored spectral range never fails to have a strong impact on the science. Radio astronomy (which covers the spectral range from about one millimetre to longer wavelengths of many meters) provides many examples. The science itself was started accidentally by Karl Jansky in 1931 when he discovered radio radiation from the Galaxy. It has developed rapidly since the late 1940s, providing an extraordinarily rich harvest of new information and broadening enormously our view of the nature of the Universe. It first demonstrated the existence of synchrotron radiation, generated by relativistic electrons spiraling in magnetic fields in our own Galaxy and others.

Quasars, pulsars, and the all-pervading 3 K black-body radiation that is the relic of the original big bang that marked the start of the expanding Universe, are all discoveries of radio astronomy. The first spectral line in the radio range (the radiation arising from neutral hydrogen at a wavelength of 21 cm) was discovered in 1951, and the first molecular line in the radio range (originating in the hydroxyl radical OH) was observed in 1963.

Since then, radio astronomy has greatly changed astronomers' views of the chemistry of the interstellar medium. Radio astronomy has shown that polyatomic molecules, earlier thought to be highly improbable if not impossible in the interstellar medium, do, in fact, abound in the dark cool clouds of interstellar space. Radio spectral lines from several dozen different polyatomic molecules, some containing up to nine atoms, have been observed.

An extraordinary feature of the radio lines of the OH molecule was discovered in the course of a series of observations made at the Hat Creek Observatory of the University of California at Berkeley during 1964–65. The Hat Creek programme called for observations of all likely radio sources in which the OH molecule might appear. Many clouds containing OH were found, but in a few cases in which

dark clouds were associated with regions of ionised hydrogen excited by hot, ultraviolet emitting stars, the behaviour of the four spectral lines in the multiplet observed was totally anomalous.

The shapes and strengths of the four lines of the multiplet seemed to have little or no relationship to one another, a situation completely out of keeping with theoretical predictions. The lines were mixtures of broad absorption and numerous sharp emission components. Subsequently, it was found that in some instances the sharp emission features were polarised (some linearly, some circularly), and that some features varied in intensity on the timescale of a few weeks. The emission components originated in regions of the dark clouds having angular diameters far smaller than could be resolved by any single radio telescope. Later observations showed that the OH emission came from areas less than 10^{-2} arcs in size.

It is an interesting historical sidelight that, while these observations were in progress at Hat Creek, similar observations were being made at the Harvard Radio Astronomy Observatory, where the same peculiarities were independently discovered. Neither group knew of the work of the other. By chance, the discovery was published first by the Berkeley investigators.

The peculiarities of the OH emission combined with the intensity of the radiation and the small signs of the sources from which it originated, were clearly inconsistent with spontaneous emission from a gas in thermal equilibrium. Amplification by stimulated emission must be taking place. Natural masers (microwave amplification by stimulated emission of radiation) in astronomical objects were thus accidentally discovered a few years after masers were invented on Earth. An interesting speculation will always remain as to how the theory of masers might have developed if the astronomical observations had come first.

In his brief 135-page monograph Professor A. H. Cook deals with the observation and interpretations of these intriguing astronomical objects. The topic has, of course, grown greatly in the 12 years since the first discovery. It is now known that not only does the OH group but also the H_2O and other molecules, show maser action. Theories of "how it works" are numerous.

In the preface to his book Professor Cook states: "I have not presumed to write a book that will sum up the subject and give an explanation. Rather I have tried to bring out what is not known; no doubt I shall provoke disagreement, and if that disagreement leads to new knowledge and insight,

much of my purpose will have been achieved. This is meant to be an interim report and a stimulus for further study." Professor Cook has very successfully achieved his goal. His summaries and concluding remarks should prove stimulating to both seasoned workers in the field and to those who may wish to enter the field.

Of the six chapters in the book, Professor Cook devotes the first two to a short account of selected spectral lines in radio astronomy and a very brief review of those principles of molecular structure and spectroscopy required for discussion of celestial masers. He illustrates the topics covered with examples from OH and H_2O sources.

The third and longest chapter provides a very adequate summary of observations of maser action for OH and H_2O sources. Here, Professor Cook has steered a straight and true course, emphasising and summarising those features that are important for a basic understanding of the topic and foregoing the very large amount of detail found in extensive research papers.

In explaining the amplification of stimulated emission (chapter 4), the author adopts a phenomenological point of view and avoids a general quantum mechanical treatment. He provides clear brief derivations or explanations of the principal aspects of astrophysical masers, such as the equations of transfer and rate, the meaning of saturated and unsaturated masers, gain, linewidth, and the like. But the author's major thrust in this chapter is to point out the gaps in current knowledge. "We do not assuredly know", he writes, "how to account for the small angular sizes of sources, the isolated distribution of individual sources, the single sense of polarisation in hydroxyl, and, again in hydroxyl, radiation in only one transition. Possibly the greatest lack is the absence, so far, of any but the sketchiest account of lasers varying with time".

After reviewing quite thoroughly in chapter 5 the many schemes that have been suggested for pumping, that is, for inverting the populations in OH and H_2O sources to produce a maser, Professor Cook goes on in chapter 6 to provide an analysis and interpretation of maser radiation. Here he attempts "to see how far the sources can be accounted for by amplification of stimulated emission and to use the analysis to set conditions that must be fulfilled by any pumping mechanism. Other clues to pumping mechanisms may be provided by the statistical properties and associations of maser sources". The chapter is a very interesting one in which the author enumerates various classes of problems

remaining to be solved; and, in some instances, he suggests bases for their solution. Observational classification schemes of OH sources are paired with theoretical pumping schemes in a most interesting way. Finally, Professor Cook tries briefly to fit celestial masers into a wider picture of the star formation process.

Celestial Masers is a book that many will find interesting and stimulating. I

highly recommend it. Unfortunately, it does contain a few typographical errors in both references and equations, but the reader will readily recognise and easily correct these very minor blemishes. □

Harold Weaver is Professor of Astronomy at the University of California at Berkeley, and was the Founder-Director of the Radio Astronomy Laboratory at Hat Creek.

Nuclear shapes and sizes

C. J. Batty

Nuclear Sizes and Structure. By R. C. Barrett and Daphne F. Jackson. (Clarendon: Oxford, 1977.) Pp. 566. £17.50.

In his classic paper on the scattering of alpha particles at large angles, Rutherford wrote: "Considering the evidence as a whole, it seems simplest to suppose that the atom contains a central charge distributed through a very small volume, and . . .". And so we had the first insight into the structure of the atom with its central charged nucleus, later shown to consist of (uncharged) neutrons and (charged) protons, surrounded by a cloud of electrons. Since then, our knowledge of the constituents and the structure of the nucleus has advanced rapidly, but this very sentence of Rutherford's also poses more detailed questions. How big is the nucleus? What shape does it have? How are the constituents and the electric charge distributed? And so on.

The study of nuclear sizes, in its widest sense, has remained since those early days at the core of nuclear physics and of studies on the structure of the nucleus. The success, or otherwise, with which nuclear models can make meaningful predictions as to the distribution of charge and nuclear matter in the nucleus has always been a sensitive test of their validity. The study of nuclear shapes and sizes too has always encompassed a very wide range of topics. The charge distribution, and hence the proton distribution, can be sensed with the electron or the muon through the electromagnetic interaction, and we now have quite detailed information available from measurements of electron scattering and with muonic atoms. Study of the matter distribution which is necessary to provide the neutron distribution, requires the use of strongly interacting probes such

as the proton, alpha particle, pion, kaon, and so on; and a much wider range of nuclear scattering and interaction processes are involved. As a result, our knowledge is much less certain and in certain cases ambiguous or downright conflicting.

It is now 16 years since the monograph on *Nuclear Sizes* by L. R. B. Elton appeared, and the intervening years have seen dramatic advances in our knowledge of nuclear shapes and sizes and in the techniques used for their study. This book by Roger Barrett and Daphne Jackson of the University of Surrey is therefore particularly welcome and provides a long needed successor to the earlier monograph.

Over the past few years, it has become clear that the different types of experiments used to look at nuclear sizes probe different regions of the nuclear matter or charge density and hence are sensitive to different parameters or combinations of parameters of the form chosen for the distribution. It has also led to the so-called 'model-independent' analyses in which there is no rigid *a priori* choice of the form for the nuclear distribution and in which the uncertainties in the derived distribution are more clearly seen.

The first chapter of the book, then, deals with these introductory ideas. Various distribution functions and form factors are defined and the ideas of radial parameters and moments introduced. Some useful comparisons are also made between the numerical parameters calculated from a range of neutron and proton distributions.

It might be concluded, so far, that all the advances of recent years have been on the experimental front. This is by no means so, and the second chapter deals with the various current theories of the nuclear matter distribution. After some introductory ideas, the single-particle model is discussed; in this model individual particles move in an averaged phenomenological nuclear potential. As this potential is, in fact, derived from the interactions of all the nucleons in the nucleus, the model lacks self-consistency. This problem is avoided by using the Hartree-Fock method; and an extensive review

of recent calculations is given. Macroscopic models in which the emphasis is on bulk nuclear properties are next discussed, particularly the Thomas-Fermi approximation and the hydrodynamical model; and the chapter then concludes by considering the especial features of calculations for deformed nuclei. Results from the various types of calculations are compared in a number of useful tables and graphs throughout the chapter.

As already mentioned, measurements of electron scattering and with muonic atoms are used to obtain information about the charge distribution in nuclei; and these topics are discussed in the next two chapters. After considering the motion of electrons and muons in an electromagnetic field, the scattering of electrons by the nuclear charge is discussed. Analyses to obtain the charge distribution using either a simple parameterised model or with a 'model-independent' method are next described, together with a brief discussion of the problems raised by analyses for deformed nuclei. Large angle magnetic scattering is also considered, followed by a discussion of dispersion, radiative and straggling corrections.

For muonic atoms, the chapter moves quickly into a discussion of 'model-independent' analyses of the X-ray spectra, the energy levels for deformed nuclei, magnetic hyperfine structure, and isotope, isomer and isotope shifts. Radiative corrections are particularly important in muonic atoms and until recently were the subject of considerable controversy when measurements for high transitions seemed to indicate a disagreement between experimental results and calculations using quantum electrodynamics. Happily, this problem now seems to be resolved and a review of these topics completes the chapter.

The use of optical and X-ray spectral methods to measure isotope and isomer shifts is only very briefly touched on in the next chapter (just ten pages long). This is unfortunate as the topic is of considerable current interest, particularly in view of the increasing use of laser techniques. Also, the following chapter on experimentally derived charge distribution shows that quite a large body of information has been obtained in this way. This latter chapter, as well as including tables on isotope and isomer shifts, also includes tables on charge distributions for a very wide range of nuclei, including deformed nuclei. Inevitably, such information rapidly becomes out of date and the serious researcher will need to go back to the original literature or to other, more extensive, data compilations. Nevertheless, the tables present a great deal of

useful information in a very compact form and include an extensive set of references to enable the reader to dig deeper.

As indicated, the study of the nuclear matter distribution involves the use of strongly interacting projectiles and the derivation of the required information from nuclear scattering or reaction experiments. These topics, which are dealt with in the next two chapters, are inevitably complicated and it is not too surprising that together these two chapters account for almost half the book.

Nuclear scattering is dealt with first. After an introduction to partial wave methods of analysing scattering from a potential and to the various approximations which are used, the formal theory and general properties of the optical model are dealt with. This is followed by a review of the information gained from analyses of nucleon-nucleus scattering. This review is split into three subsections, covering different energy regions, and includes many tables of optical model potentials and parameters. An equally complete review of the scattering of composite projectiles and of the nuclear size information which can be obtained from experiments then follows.

The chapter on nuclear reactions starts with an extensive discussion of inelastic scattering both to resolved final states and then to summed final states; the former gives information principally about particular spectroscopic states and the latter about average nuclear properties. There follows descriptions of the theory and analysis of transfer, knock-out and photoneuclear reactions, and finally of alpha decay. Inevitably, any discussion of nuclear reactions also involves problems related to nuclear reaction mechanisms and to the structure of nuclei. The chapter does, however, pay particular attention to the information about nuclear sizes and other closely related nuclear properties which can be obtained in this way.

It was originally thought that measurements on hadronic atoms would be capable of giving clear information on nuclear matter distributions. It turns out, however, that here nature has not been particularly kind. In the case of pionic atoms, the interaction of the pion with the nucleus is complicated by the presence of dominant p -wave processes, whereas for kaonic atoms the resonance in the kaon-proton system again complicates the analysis of experimental data. It is still hoped, however, that, with our growing understanding of these interactions, it may be possible to obtain nuclear size information; these topics therefore are discussed in chapter 9 of the book.

The final chapter concentrates on processes capable of giving information about the neutron distribution directly rather than through separate measurements of the proton and matter distributions. Available methods for looking at the excess neutrons include studies of Coulomb energy differences or of charge-exchange reactions. Alternatively, the scattering of pions can be measured at energies at which they interact preferentially with neutrons. Pion production, or the interactions of stopping kaons, can also be used, since in both these cases certain processes can only occur through interactions with neutrons.

This chapter-by-chapter review will have indicated the very wide range of topics covered by the book, all of which are related in some way to the question of nuclear sizes. In view of this broad coverage there is inevitably some unevenness in the treatment of the various topics. How much this matters is a matter for individual judgment, which each reader will have to decide for himself in the light of his own special interests.

The book contains a large number of tables and figures of useful data. As already mentioned, a few of the tables will rapidly become out of date. Nevertheless, they comprise a useful collection of information related to nuclear sizes. The book also contains a list of approximately 1,500 references which fill the last 50 pages of the book and which are frequently referred to throughout its pages. This makes the volume particularly useful to the specialist, who will wish to refer to the original literature. It does, however, create the air of an extended review article rather than that of a definitive text which might appeal more to the student approaching the subject for the first time. To some extent, this is due to the authors' determination to include the most recent developments.

Finally, as the authors themselves point out, the past few years have seen an enormous growth in our knowledge of nuclear sizes and in the range of experiments used for their study. The book provides an excellent review of the situation as it was at the time of writing in 1975. The next few years are likely to see an equally rapid progress in both experimental work and in theoretical calculations. It is also likely to see an ever-increasing range of methods in atomic, nuclear and particle physics being used to study the sizes and shapes of nuclei. □

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review article

Nature of lethality of *t* mutations in embryos

Linda R. Wudl* Michael I. Sherman* & Nina Hillman†

Mutations in the genes of the T complex in mice can lead to death at various stages of embryogenesis. It has been suggested that the T genes specify cell surface antigens which are essential for normal organisation of certain cells within the embryo. But, there is now evidence that at least some of these T complex mutations are simply generalised cell lethals.

THE set of genes in the mouse known as the 'T complex' has been studied extensively for many years. However, there is still disagreement on both the genetics and the biochemistry of this system. The confusion arises in attempts to explain how apparently single genetic alterations can result in multiple phenotypic effects. Such alterations in the T region, conventionally referred to as recessive (*t*) mutations, may cause (1) death of homozygous embryos at characteristic stages, (2) taillessness when combined with the Brachyury (*T*) mutation in a compound heterozygote (*T/t*), (3) non-Mendelian inheritance from the male parent, (4) suppression of recombination over a rather large region of chromosome 17 (extending, in the case of *t^{w5}*, from the centromere to well beyond the H-2 region) and, (5) male sterility when combined with other, complementary, *t* mutations (*t^x/t^y*)^{1,2}. Present knowledge of the genetics of the T region indicates that although a single mutagenic event may have resulted in what is called the '*t* mutation', a large portion of the genome and multiple loci are affected^{1,2}. We shall discuss only the lethal effects of mutation in the T region and the relationship between the *t* genes (or gene) and the programme for early embryonic development.

Two theories have been put forward to explain the lethality of *t* mutations. The first, and most widely quoted, of these proposes that the primary lesion is an alteration at the surface of certain embryonic cells which prevents the cell-cell recognition and interaction required for tissue formation ('organisational failure' hypothesis)¹⁻⁶. The affected embryo becomes disorganised and is resorbed in the uterus. The second theory is simply that, at least for some *t* mutations, all cells of the embryo are adversely affected and that death of the embryo is due to generalised cell lethality⁷⁻¹¹.

In earlier studies¹¹, we tested the organisational failure hypothesis by monitoring the behaviour of mutant embryos *in vitro*. In our culture conditions^{12,13}, the organisation and interactions of different cell types are less important for cell survival than they are *in utero*. This is documented by our success in establishing a number of cell lines from cultured blastocysts even though several cell types (for example, embryonic ectodermal derivatives) either degenerate soon after formation or are never produced. In fact, the cell types that develop most reliably in our cultures are extraembryonic endoderm and trophoblast¹³; these are the very cell types that seem to survive longest *in utero* in conceptuses homozygous for every lethal *t* allele² with the possible exception of *t^{w73}* (ref. 14). Consequently, our *in vitro* techniques should have enabled us to recover those cells from homozygous *t*-mutant embryos that are presumably not directly affected by the mutation. However, when we tested homozygous *t^{w5}* mutant embryos¹¹, we found that none of the cells was capable of surviving *in vitro*, and indeed, most of the

cells had died at the time of the normal lethal period for *t^{w5}/t^{w5}* embryos *in utero* (7-10th day of gestation¹⁵). Furthermore, when pairs of embryos are combined to form chimaeras, homozygous mutant cells in the mosaic embryos do not seem to be rescued by contact with their normal neighbouring cells; in other words, the *t^{w5}* mutation behaves as a generalised cell lethal and not as an organisational lethal¹¹.

Investigators studying the extrauterine development of embryos bearing two other recessive *t* mutations, *t⁶* (ref. 16), which causes death earlier than *t^{w5}*, and *t^{w18}* (ref. 17), which leads to embryonic failure at later stages, have come to conclusions different from those we have drawn on the basis of experiments with *t^{w5}*. Erickson and Pedersen¹⁸ reported that the inner cell mass (ICM) of presumptive *t⁶/t⁶* blastocysts showed a rapid degeneration *in vitro* while the trophoblast remained viable. They concluded, therefore, that the adverse effects of the *t⁶* mutation were selective for the ICM moiety of the embryo. Artzt and Bennett¹⁹ transplanted 9th d presumptive *t^{w18}/t^{w18}* embryos under the testis capsule of adult mice and proposed, from analyses of the resultant growths, that ectodermal derivatives of these embryos were capable of surviving whereas mesodermal elements failed to develop. We report here the results of our studies on the development of *t⁶/t⁶* blastocysts both in culture and in an ectopic site. These studies were undertaken to determine whether any cells from the mutant embryos stand a better chance of survival in these experimental conditions than they do *in utero*. We have also assessed the behaviour of *t^{w18}/t^{w18}* embryos *in vitro*.

Behaviour of *t⁶/t⁶* embryos *in vitro*

An analysis of the male transmission frequency of the *t⁶* mutation is shown in Table 1a. By the use of these data, we can deduce the percentage of blastocysts with the *t⁶/t⁶* genotype in our cultures (Table 1b). The ICM cells in *t⁶/t⁶* embryos behave under our culture conditions as observed by Erickson and Pedersen¹⁸ in their experiments: by the 7th EGD (equivalent gestation day, that is the age of the embryos had they been left *in utero*), all ICM cells in 35% of the embryos had disappeared, suggesting that more than 80% of the *t⁶/t⁶* embryos contained only trophoblast. By the 8th EGD, all homozygous mutant embryos seem to have lost their ICM (Table 1c).

When we examined the trophoblast moiety of presumptive *t⁶/t⁶* embryos, however, we could see that they too had been affected by the mutation. In all control blastocyst outgrowths containing an ICM, and in the occasional blastocyst outgrowth which had lost its ICM by the 8th EGD, the trophoblast cells had proceeded to polyploidise and become giant. By contrast, the trophoblast cells of presumptive *t⁶/t⁶* blastocyst outgrowths did not become giant².

In a subsequent series of experiments, we generated trophectodermal vesicles from disaggregated blastomeres of normal and *t⁶/t⁶*

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Table 1 Transmission frequency distortion of the t^6 allele and behaviour of blastocysts bearing the t^6 mutation in culture

a Transmission frequency of the t^6 mutation							
Genotype at T region of animals mated female male (+/+) × (T/t^6)		Number of offspring	Number of offspring with normal tails		Transmission frequency of t^6		
		1,656	1,318		79.6%		
b Expected proportions of genotypes in mixed embryo populations							
	Genotype at T region of animals mated female male	% of embryos with genotype					
Cross	(+/+) × ($+/t^6$)	+/+	$+/t^6$	t^6/t^6			
Control	(+/+) × ($+/t^6$)	20.4	79.6	—			
Experimental	($+/t^6$) × ($+/t^6$)	10.2	50.0	39.8			
c Survival <i>in vitro</i> of ICM cells in embryos carrying the t^6 mutation							
Genotype at T region of animals mated	Number of blastocysts cultured	7th EGD*		% of ICMs surviving 8th EGD		11th EGD	
		observed	expected†	observed	expected†	observed	expected†
(+/+) × ($+/t^6$)	432	96.3	—	95.4	—	91.2	—
($+/t^6$) × ($+/t^6$)	503	65.2	58.0	54.5	57.4	47.4	54.9

In *a*, to determine transmission frequency, wild-type (SWR/J) females were mated with tailless males. Offspring of genotype +/ t^6 were normal-tailed (and were used in the experiments in *c*), while T/+ progeny were short-tailed. Part *b* of the table, based on the data in part *a*, gives the expected proportions of genotypes of the embryos used in *c*. In *c*, embryos were removed at the blastocyst stage, on the fourth day of pregnancy, washed, and cultured individually in NCTC-109 medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics as described by Wudl and Sherman¹¹. They were scored for the presence or absence of inner cell mass derivatives with phase contrast optics.

*EGD, equivalent gestation day, that is, the age of the embryos had they been left *in utero*.

†Expected values if ICM cells are absent in all t^6/t^6 embryos and if ICMs of heterozygous and wild-type embryos in the control and experimental populations survive with the same frequency.

mutant embryos^{20,21}. Generally, at a time when control embryos have formed mature blastocysts, trophoctodermal vesicles contain only a single layer of trophoctoderm cells, that is they are devoid of ICM cells. When trophoctodermal vesicles are placed in the appropriate culture medium, they become attached to the substratum and give rise to outgrowths of trophoblast cells which are capable of differentiation as determined by morphological and biochemical criteria^{21,22}. We studied one of these criteria, polyploidisation, in outgrowths from normal and presumptive t^6/t^6 trophoctodermal vesicles by monitoring the nuclear diameters of trophoblast cells. (We have shown elsewhere^{23,24} that DNA content in polyploid trophoblast cells is proportional to nuclear size.) Approximately 40% of trophoctodermal vesicles from the experimental cross (see Table 1) gave rise to trophoblast cells which possessed few or no giant nuclei (for example, Fig. 1, *a, c, e, g*) before death on or about the 12th EGD. On the other hand, in the control cross, which yields only heterozygous and wild-type embryos, all trophoctodermal vesicles gave rise to polyploid trophoblast cells by the 8th EGD (for example, Fig. 1, bottom photos). Non-mutant trophoblast cells survived longer than the arrested cells in the t^6/t^6 trophoblast outgrowths (Fig. 1*g, h*). A detailed quantitative analysis of these experiments, to be presented elsewhere (L.W. and M.I.S., in preparation), indicates that trophoctodermal vesicles from normal (+/ t^6 and +/+) embryos in the experimental cross polyploidise to the same degree as their counterparts in the control cross.

Table 2 Incidence of haemorrhagic nodule formation in kidneys bearing implants of blastocysts from control and experimental crosses

Cross	No. of blastocysts implanted	No. of kidneys containing nodules	% observed	% expected
Control (+/+) × (+/ t^6)	68	43	63	—
Experimental (+/ t^6) × (+/ t^6)	63	28	44	38

Fourth day blastocysts were removed from uteri and placed in culture. Within six hours, blastocysts were transplanted individually under the capsule of the left kidney of adult male siblings. Kidneys were scored macroscopically for the presence of haemorrhagic nodules. The percentage of growths observed in the control and experimental crosses was significantly different by χ^2 analysis ($\chi^2 = 7.25$, $P < 0.01$). The percentage of growths in the experimental cross observed and expected (assuming that all t^6/t^6 embryos will fail to produce growths and that the transmission frequency for the t^6 allele is 79.6%) was not significantly different ($\chi^2 = 0.74$, $P = 0.5$).

The obvious interpretation of these results is that trophoblast cells are affected by the t^6 mutation, contrary to the report of Erickson and Pedersen¹⁸; furthermore, the arrest of t^6/t^6 , but not normal, cells in these experiments renders it unlikely that mutant trophoblast cells fail through a lack of interaction with other cells, since there are no cells other than trophoblast in trophoctodermal vesicle outgrowths. Organisational failure is thus an inadequate explanation of the adverse effects of the t^6 , as well as the t^{w5} , mutation.

We have now begun to monitor t^6 mutant trophoblast cells for production of other markers of differentiation. We found (L.W. and M.I.S., in preparation) that presumptive t^6/t^6 trophoblast cells possess plasminogen activator activity during the period in which normal trophoblast cells produce and secrete this enzyme^{22,25}. They also convert pregnenolone to progesterone, indicating that they possess the enzyme Δ^5 , 3 β -hydroxysteroid dehydrogenase, as do normal trophoblast cells^{24,26}. These results suggest that the individual elements of the developmental programme of trophoblast cells may be regulated separately, and that mutation in the T region may interfere selectively with some developmental processes, but not others.

Behaviour of t^6/t^6 embryos in an ectopic site

Although all cells from t^6/t^6 embryos are adversely affected by the mutation in culture, some cells from presumptive t^{w18}/t^{w18} embryos implanted under the testis capsule survive for times longer than the gestation period¹⁹. It is, therefore, possible that t^6 and t^{w18} mutations differ, either in type or in severity. Alternatively, one might argue that ectopic sites provide a rich environment for growth of embryonic cells, allowing proliferation of genotypically mutant cells that do not require wild-type T-gene products for survival and growth, whereas our culture conditions might be detrimental to all homozygous t -mutant cells. To distinguish between these possibilities, we implanted blastocysts from t^6 control and experimental crosses under kidney capsules of immunologically appropriate host mice, and inspected the kidneys of these animals for ectopic growths 14 d later (Table 2). We found that the blastocysts from the experimental cross gave rise to a significantly lower proportion of growths visible under the dissecting microscope as haemorrhagic nodules when compared with blastocysts from the control cross. In fact, the observed number of growths was not significantly different from that expected if all t^6/t^6 embryos had failed to survive the 14-d period under the kidney capsule. The size of the nodule varied from one

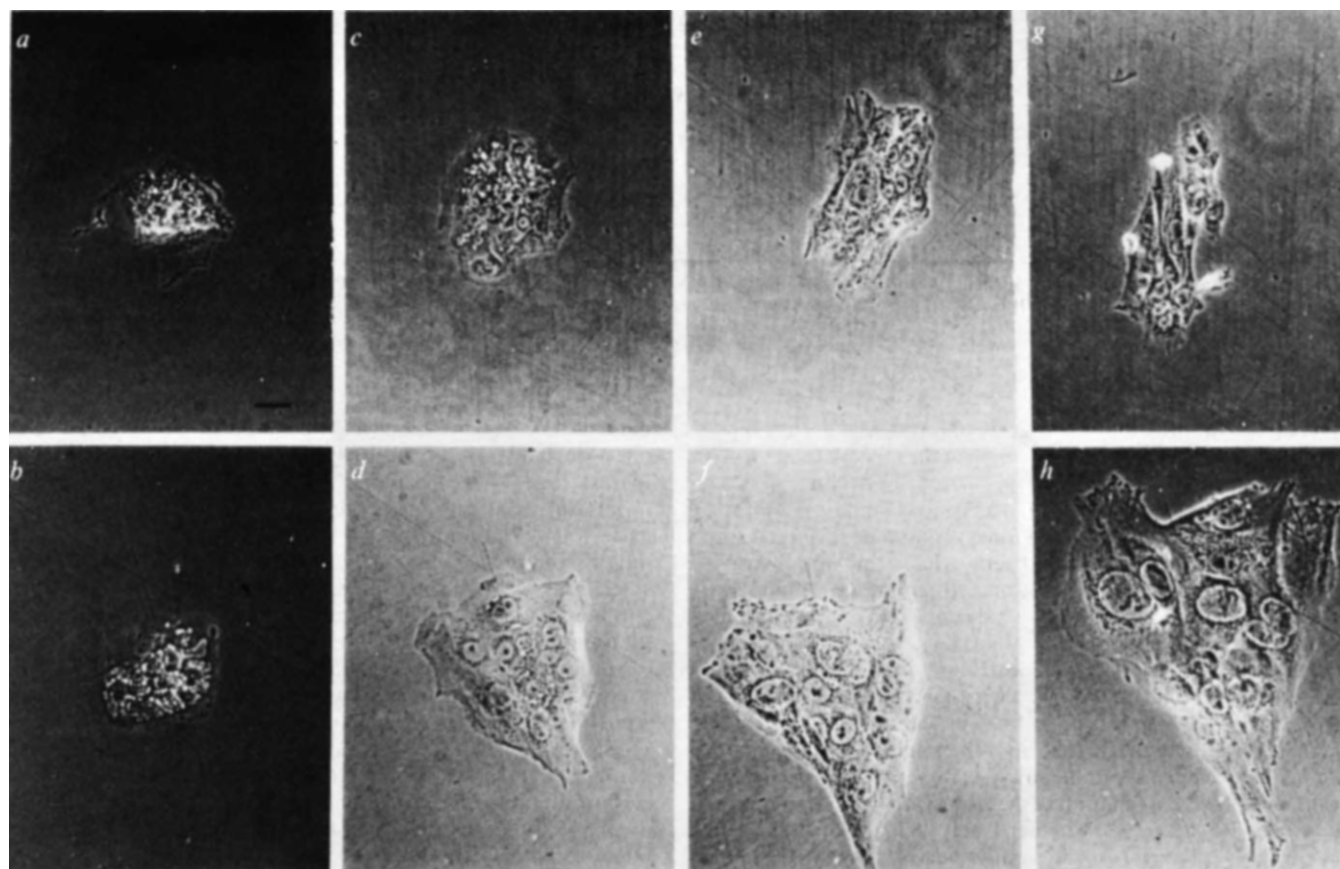


Fig. 1 Behaviour of mutant (t^6/t^6) and normal ($+/+$, $+/-$) trophoblast cells developing from trophoctodermal vesicles. Trophoctodermal vesicles were generated by disaggregating 4- and 8-cell embryos²¹ from control ($+/+ \times +/+$) and experimental ($+/- \times +/+$) crosses and culturing individual blastomeres from 4-cell embryos and pairs of blastomeres from 8-cell embryos in Whitten's modified ovum culture medium³³. After 48 h (5th EGD), structures scored as trophoctodermal vesicles were placed individually in serum-supplemented NCTC-109 medium¹¹. Trophoblast cells outgrowing from a presumptive mutant (a, c, e, g) and a normal (b, d, f, h) trophoctodermal vesicle were photographed sequentially on the 7th (a and b), 8th (c and d), 9th (e and f) and 11th (g and h) EGD using phase contrast optics. Magnification is the same in all photographs. Scale bar (in a), 50 μ m.

implant to the next, but the range of sizes was the same in the experimental and control crosses.

Approximately half of the kidneys receiving implants of blastocysts were processed histologically and analysed by light microscopy (Table 3) to determine whether we might have missed viable t^6/t^6 ectopic growths either because they were much smaller than those caused by normal embryos or because they were devoid of functional trophoblast cells and, therefore, non-haemorrhagic. In either case, the growth might not be discernible when the kidneys were examined macroscopically. The histological studies also enabled us to compare growths from experimental and control blastocysts to determine whether the growths from one cross might contain cell types not present in growths from the other. We found, however, that virtually all kidneys judged to be positive by the presence of a macroscopic haemorrhage contained extravasated blood, large numbers of giant trophoblast cells, both mononuc-

leated and multinucleated, and extraembryonic endodermal cells. In several of the explants, the endodermal cells were associated with an amorphous eosinophilic material that has been described by other investigators²⁷⁻²⁹ as Reichert's membrane.

No other recognisable embryonic tissues or organs were found in any of the haemorrhagic nodules at the time of autopsy. However, the extensive growth of trophoblast cells and identification of extraembryonic endoderm attest to the presence in the growths, at least initially, of ICM cells^{29,30}. In all of the growths, some scattered necrotic cells were present, and these might have been of ICM origin. Fibroblastic cells were commonly observed around the periphery of the growths, and, less frequently, in the central blood cavity. It is assumed that these cells were of maternal origin. In about 40% of the cases in both experimental and control crosses, the trophoblast cells were actively invasive and phagocytosed kidney cells could be seen in the giant cell cytoplasm; the

Table 3 Histologic analyses of kidneys receiving implants of normal and mutant (t^6/t^6) blastocysts

Cross	No. analysed	Macroscopic positives		Macroscopic negatives	
		No. containing trophoblast and extra-embryonic endoderm cells	No. containing Reichert's membrane-like material	No. analysed*	No. containing growths
Control ($+/+$) $\times$ ($+/-$)	16	15†	7	15	0
Experimental ($+/-$) $\times$ ($+/-$)	17	17	6	15	1

After the kidneys referred to in Table 2 were inspected macroscopically for the presence of growths, they were placed in neutral buffered formalin, embedded in paraffin and serially sectioned (8 μ M). After dehydration in a graded series of alcohol, the sections were stained with Delafield's haematoxylin and eosin.

*Entire kidneys were prepared as described above and every section was scanned with low power optics.

†One nodule, which was very large, was only partially sectioned. Those sections inspected contained only blood cells and extraembryonic endoderm cells.

Table 4 Survival *in vitro* of embryos carrying the t^{w18} mutation

Cross	Expected % of homozygous mutant embryos	No. of blastocysts cultured	% of blastocysts surviving 22–24th EGD	
			observed	expected*
Control (+/+) × (+/ t^{w18})	0	404	55.0	
Experimental (+/ t^{w18}) × (+/ t^{w18})	28.7†	402	51.5	39.2

The +/ t^{w18} mice were generated by selecting normal-tailed offspring from the cross of SWR/J females (wild-type in the T region) with T/ t^{w18} males (obtained from Dr Karen Artzt, Sloan Kettering Institute for Cancer Research).

*Expected incidence if all t^{w18}/t^{w18} embryos fail to survive the gestation period *in vitro*.

†The proportion of homozygotes is low for t^{w18}/t^{w18} embryos because the t^{w18} allele does not show substantial transmission frequency distortion¹⁷.

remainder of the growths did not seem to be invasive at the time of autopsy, but rather caused a depression of the host tissue at the site of transplantation. Of 30 kidneys scored as negative macroscopically, all but one proved to be negative upon microscopic examination. The exceptional kidney contained a large, subcapsular growth, which was non-haemorrhagic and non-invasive. It lacked trophoblast giant cells and extraembryonic endoderm, containing instead epithelial cells which appeared to be embryonic in nature, some of which were arranged cylindrically, reminiscent of neural tubes. Although this kidney was in the 'experimental' group, we have no way of knowing whether it had received a t^6/t^6 , a +/ t^6 or a +/+ blastocyst. In fact, we cannot say for certain whether this growth originated from the implanted blastocyst or whether it developed spontaneously.

The most likely explanation of these observations is that normal (+/+, +/ t^6) blastocysts, whether from the experimental or the control cross, give rise to the same kinds of growth under the kidney capsule, while all cells from t^6/t^6 blastocysts die within the two week incubation period.

Behaviour of t^{w18}/t^{w18} embryos *in vitro*

As the results in Table 4 illustrate, over 50% of individually cultured control blastocysts from a population consisting of approximately equal numbers of +/+ and +/ t^{w18} embryos give rise to cells which survive longer than the normal gestation period. Similarly, blastocysts from the experimental cross show approximately the same survival frequency. The implication from these results is that at least some cells from many of the cultured t^{w18}/t^{w18} blastocysts are viable well beyond the normal period of lethality of homozygous mutant embryos *in utero* (that is about the 9th day of gestation), a finding consistent with the ectopic implant studies¹⁹. Inspection of the cultures after three weeks (and at later times) did not reveal the presence of cells in the experimental cross population which were morphologically distinguishable from cells in the control cross population. Several of the single blastocyst-derived cultures from both the experimental and control crosses have been maintained *in vitro* for several months, and experiments are in progress to determine their genotype.

Conclusions

According to the organisational hypothesis, t mutations "affect discrete steps in development by impairing the differentiation of particular embryonic cell types"¹⁴, presumably by interference with "processes necessary to cell-cell interaction and determination"¹¹. The experiments described above, as well as others already published^{7–11}, are inconsistent with this hypothesis, at least insofar as three classes of early-acting t mutations (t^{12} , t^6 , t^{w5}) are concerned. In each case, all cells of the embryo, rather than specific cell types, are adversely affected. The facts that cells from t^6 -mutant embryos fail to survive in ectopic sites as well as in culture, and that some cells from t^{w18} -mutant embryos seem to survive under both conditions, reduces the possibility that the observed generalised effect of early-acting t mutations is due to inadequate culture conditions. Our observations with t^6 -mutant embryos also argue against impairment of all differentiation in affected cells, since t^6/t^6 trophoblast cells, clearly abnormal in that they fail to endoreduplicate like their normal counterparts,

nevertheless, express characteristic differentiation markers (plasminogen activator, Δ^5 , 3 β -hydroxysteroid dehydrogenase). Furthermore, the block to endoreduplication is unlikely to be related to improper cell-cell interaction, either self or non-self, since we have found that normal trophoblast cells are capable of initiating and continuing endoreduplication, even when isolated as single cells (S.B. Atienza and M.I.S., unpublished observations).

It is difficult to place the observed alterations in surface antigens of t -mutant sperm³ and embryos^{3,1} into perspective with our observations because unequivocal evidence has not been presented to indicate whether such antigens are primary t -gene products, secondary effects resulting from mutation in the T region, or products of genes closely linked to the T complex which seem to be part of it due to suppression of recombination. Of course, surface alterations can lead to cell lethality in ways other than those involving faulty cell-cell interactions; for example, they might interfere with intracellular processes such as those related to replication and the cell cycle³².

We do not yet know why some cells in embryos homozygous for later-acting t mutations are able to survive and proliferate while all cells in early-acting t -mutant embryos die. It may be that some cells at advanced stages of differentiation do not express the altered product(s) or require the wild-type product(s) of the T-complex genes. We stress, however, that there is a danger in making comparisons between the various classes of lethal t mutations until more is learned about the number and nature of genes in the T complex.

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articles

Transformation of the state of nitrogen in diamond

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High temperature-high pressure annealing experiments on diamond transform type IB dispersed nitrogen into type IA aggregate nitrogen. The activation energy for the transformation is approximately 60 kcal mol^{-1} (2.6 eV). An estimate is given for the diffusivity of nitrogen in diamond for this transformation

NITROGEN impurity is found in substitutional lattice positions in most natural and synthesised diamonds, but its usual state differs in the two types of diamond. Most natural diamonds that contain nitrogen have it in a form that is inactive to electron paramagnetic resonance (EPR) and these diamonds have been classified type IA. In the past this form of nitrogen has been pictured as localised in the platelets characteristic of type IA crystals, but evidence suggests the nitrogen may occur in clusters of 10–13 atoms¹ or substitutional nitrogen pairs² rather than in platelets. Nitrogen in synthesised diamonds is EPR active and atomically dispersed. These diamonds have been placed in the type IB classification.

A few natural diamonds are type IB or a mixture of types IA and IB. No synthesised diamonds with wholly type IA properties have been made, but some type IA characteristics in certain synthesised diamonds have been reported^{3,4}.

The existence of the two diamond types poses questions about their relative stability and whether the stable type may be formed by a high temperature-high pressure treatment in the laboratory. If successful, this might provide an insight into the history of natural diamonds and give important information for understanding their growth mechanisms. For example, Klyuev *et al.*³ and Evans⁵ suggested that nitrogen deposits in the atomically dispersed state (type IB) in natural diamond during growth, with the type IA state forming during the long residence in the high temperature environment deep within the earth. Is this hypothesis consistent with experimental data and could approximate residence times be ascertained?

Our experiments have indicated high temperature-high pressure treatment transforms type IB nitrogen into type IA nitrogen. This has provided information about the reaction rates for the transformation IB to IA and the diffusivity of nitrogen in diamond. Some implications are drawn from the data about the growth of natural diamonds. More experiments should clarify some old questions about platelets, X-ray spikes, and gem manufacturing.

Experimental methods

The difficult experimental conditions necessary for transforming nitrogen states in diamond are dictated by the extraordinary refractory nature of diamond as shown by its high melting point and resistance to plastic flow. The melting point is about 4,000 K at 150 kbar (ref. 6) and no plastic yielding occurs below about 2,073 K (ref. 7). Hence, any process requiring lattice diffusion of nitrogen could not be expected to proceed rapidly below temperatures of about 2,000 K. These experiments were conducted at this temperature level under pressures in the range 55–65 kbar to prevent graphitisation of the diamonds.

The GE 'Belt' pressure apparatus⁸ was used to provide the

pressurised environment for the diamond heat treating experiments. The crystals to be heated, 1–2 mm in size, were placed in a small cavity in the centre of a 3.17 mm diameter graphite rod (Fig. 1). The temperatures reached in terms of power input were measured several times with a 0.127 mm Pt–Pt 10% Rh thermocouple. Temperatures in terms of power input were consistent to within about $\pm 75^\circ$ at the 2,273 K level. Part of the variation from one test to another was due to the differing cross sections of the crystals used. This variation influenced the heating current density in the graphite rod at the diamond where its cross section was reduced by different amounts due to the presence of diamond of different sizes.

Crystals were treated at temperatures between 1,873 K and 2,273 K, usually for 30 min at a time, at pressures near to or within the region for the thermodynamic stability of diamond at the treatment temperature, 55–65 kbar.

The analytical techniques used to monitor the conversion of the nitrogen from the IB to IA state were dictated by the characteristic properties of each state. Type IA and type IB nitrogen each induce their own characteristic ultraviolet, visible, and infrared absorptions: type IA nitrogen is apparently EPR inactive but type IB nitrogen is EPR active. Many type IA diamonds are colourless whereas most type IB crystals are canary yellow.

Quantitative measurements of types IA and IB nitrogen concentrations depend on the infrared absorption intensities of bands at $1,280 \text{ cm}^{-1}$ for the type IA nitrogen and at $1,130 \text{ cm}^{-1}$ for type IB nitrogen. These two band systems have been closely correlated with nitrogen content by Kaiser and Bond⁹ for type IA nitrogen and Chrenko, Strong and Tuft¹⁰ for type IB nitrogen. Absorption spectra recorded for each crystal before and after heat treatment provided information on the amounts of each type of nitrogen present and the total in the crystal in these two states. Any change in this nitrogen total would indicate either a change in the amount of nitrogen in the crystal or a transformation to some other state not yet identified.

EPR was used in a semi-quantitative manner. The decrease in integrated intensity (amplitude $\times$ width²) of the centre line of the dispersed nitrogen hyperfine triplet was a measure of the decrease

Fig. 1 Sample holder for diamond used in the high pressure apparatus. The alumina liner protected the pyrophyllite sample holder from the high core temperature. The thermocouple was used in several experiments for calibration of temperature in terms of power input. For most annealing experiments the thermocouple was left out.

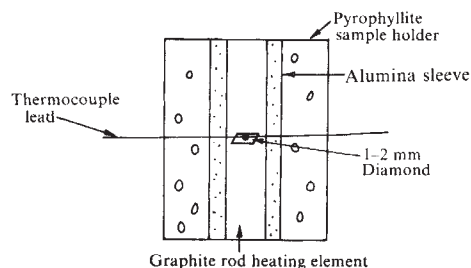


Table 1 EPR, infrared, and ultraviolet-visible data

Crystal*	<i>T</i> (K)	Total time at <i>T</i> (min)	EPR		<i>F</i> †	Infrared		Ultraviolet-Visible	
			<i>F</i> †	Type IB nitrogen‡ (p.p.m.)		Type IA nitrogen§ (p.p.m.)	IA and IB nitrogen (p.p.m.)	% Transmission at 400 nm	415 nm Absorption line
N-1	—	—	1.00	96	1.00	37	133	~3	None
S-1	2,173	30	0.38	42	0.44	93	135	~7	Present
	—	—	1.00	365	1.00	0	365	~0	None
	2,173	30	0.32	128	0.35	234	362	~5	Present
S-2	2,173	60	0.15	74	0.20	288	362	~5.3	Stronger
	—	—	1.00	237	1.00	0	237	~0	None
	1,973	30	0.79	180	0.76	24	204	~0	None

*N, natural diamond; S, synthesised diamond; N-1 was a mixed IA-IB crystal originally

†*F*, amount of type IB nitrogen remaining/original amount of type IB nitrogen

‡From 1,343 cm⁻¹ absorption; see text.

§From 1,280 cm⁻¹ absorption.

in dispersed nitrogen content. The intensities were normalised to unit intensity for the untreated crystal.

The ultraviolet and visible absorptions were not very useful for quantitative measurements because the absorption occurs in a poorly defined absorption tail. The increased transmission in the 350–500 nm region and emergence of the 415.2 nm zero phonon line of the N-3 absorption system, however, were characteristic of the transformation from type IB to IA diamond. These latter measurements therefore, confirmed that the type IB to type IA transformation was occurring.

Results

After heat treatment, the deep yellow colour of type IB crystals had faded significantly. Quantitative measurements on their spectral absorption and EPR characteristics yielded data on the transformation from IB to IA that are summarised in Table 1.

Infrared absorption spectra for a synthesised type IB crystal, before and after heat treatment at 2,173 K for 30 min, are shown in Fig. 2. A natural type IA crystal absorption spectrum is included for comparison. The partial conversion of the type IB crystal to type IA is evident.

The quantitative measurements from infrared absorption data showed that concentrations of type IB nitrogen decreased while the concentrations of type IA nitrogen correspondingly increased. The total nitrogen remained constant. Measurements of type IB nitrogen were actually made using the absorption at 1,343 cm⁻¹, which is correlated with the 1,130 cm⁻¹ absorption, since the 1,130 cm⁻¹ absorption becomes difficult to resolve as it weakens while the 1,343 cm⁻¹ absorption remains sharp and distinctive. Type IA nitrogen concentrations were obtained from its 1,280 cm⁻¹ absorption. The EPR data showed close agreement with infrared absorption data on decreases in concentrations of type IB nitrogen. The ultraviolet-visible data indicated increased transmission in the 350–500 nm region with the transformation IB to IA nitrogen, in agreement with the quantitative measurements on the transformation. In the samples wherein sufficient conversion had taken place, the 415 nm zero phonon line of the N-3 system of type IA diamonds appeared, confirming the previous evidence on the nature of the transformation.

Heat treatment of type IB synthesised crystals at 2,273 K produced only the type IA group A bands characterised by the absorption at 1,280 cm⁻¹. No evidence was seen for the group B bands, whose strongest absorption is at 1,175 cm⁻¹. Also, heat treatment at 2,273 K of a natural diamond having both the 1,280 cm⁻¹ and 1,175 cm⁻¹ group A and group B absorptions produced no shift in relative intensities of either band.

Quantitative measurements on the extent of the reaction in terms of time and temperature indicated that the transformation was controlled by second order diffusion kinetics obeying the rate equation:

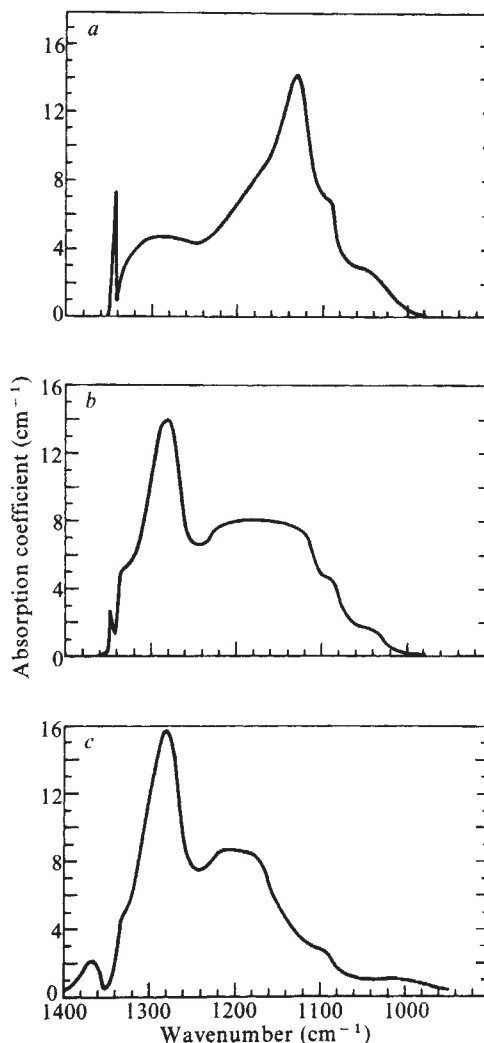
$$kt = 1/c - 1/c_0$$

in which *k*, the rate constant, was expressed as min⁻¹ p.p.m.⁻¹; *t* is the time in min; *c*₀ the initial concentration of type IB nitrogen in

p.p.m. and *c* the concentration of type IB nitrogen remaining after heat treatment. In Fig. 3, 1/*c* is plotted against *t* for the data at 2,173 K which gives *k* = 1.74 × 10⁻⁴ min⁻¹ p.p.m.⁻¹.

An Arrhenius plot for all of the reaction rate data is shown in Fig. 4. The scatter in the data points may be attributed in part to uncertainties in temperature measurement and to the fact that nitrogen can be unevenly distributed within diamond crystals. During growth, nitrogen has a strong tendency to collect in streaks

Fig. 2 Infrared absorption spectra for *a*, a type IB synthesised diamond as grown; *b*, the same diamond after a heat treatment for 30 min under pressure at 2,173 K; *c*, a typical type IA natural diamond, for comparison purposes.



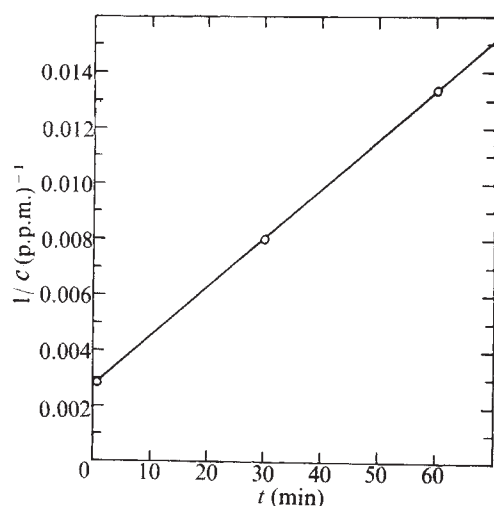


Fig. 3 A plot of $1/c$ against time in min for a type IB diamond run at 2,173 K. c is the concentration of type IB nitrogen in p.p.m. A value of $k = 1.74 \times 10^{-4} \text{ min}^{-1} \text{ p.p.m.}^{-1}$ is obtained from this data.

and patches along certain crystal planes instead of distributing itself uniformly. The infrared and EPR measurements do not differentiate between concentrated and evenly distributed nitrogen. They measure only the total dispersed nitrogen present. Because the apparent reaction rate is proportional to the concentration of dispersed nitrogen the reaction in a concentrated nitrogen streak would be seen as running much faster than it would if the nitrogen were more evenly dispersed.

The reaction rate may also be influenced by the presence of dislocations. Conceivably, certain types of dislocations would facilitate the diffusion of nitrogen in diamond thereby speeding up the formation of type IA nitrogen.

From the Arrhenius plot in Fig. 4, the activation energy for rate constant was found to lie within the range 48 to 73 kcal mol⁻¹. Using the equation $k = A \exp(-E/RT)$, assuming an activation energy for the reaction rate of 60 kcal mol⁻¹ (2.6 eV) and a k value of $1.74 \times 10^{-4} \text{ min}^{-1} \text{ p.p.m.}^{-1}$ at 2,173 K, a set of k values for the temperature range 1,573 to 2,273 K was obtained. A table of times for the reaction to reach 50, 99.0, and 99.9% completion at temperatures between 1,573 and 2,273 K was then prepared (Table 2) for an assumed initial concentration of type IB nitrogen of $c_0 = 500 \text{ p.p.m.}$

These experiments provide an opportunity to obtain some rough estimates on the diffusivity of nitrogen in diamond for the type IB to type IA reaction. This was done by assuming type IA nitrogen to be in the form of clusters and making certain other assumptions about the size and number of clusters formed, as well as the concentration gradient of dispersed type IB nitrogen between clusters.

The calculation for the diffusivity is based on Fick's first law, $\Delta N = D(dc/dx)At$; in which ΔN is the number of atoms of

nitrogen in a 1 cm³ diamond which are collected into clusters, D is the diffusivity in cm² s⁻¹, dc/dx is the concentration gradient expressed as number of atoms $\times \text{cm}^{-4}$, A the effective diffusion area in cm², and t the time in s.

The effective diffusion area, A , was taken as the area of one 13 atom cluster multiplied by the average number of clusters present during the initial 30-min anneal. One cluster was estimated to have the area of a $4 \times 10^{-8} \text{ cm}^2$ cube. The example used for calculating D was taken from Table 1 in which a crystal (S-1) having 365 p.p.m. type IB nitrogen originally was heated for 30 min at 2,173 K and then found to have its type IB nitrogen reduced to 128 p.p.m. Hence, in a 1 cm³ crystal the number of cluster nitrogen atoms, ΔN , would have been 42×10^{18} . The average number of clusters was 10^{18} and the cluster spacing was 10^{-6} cm . dc/dx was assumed to be $42 \times 10^{18} \text{ cm}^{-3}$ divided by 1/2 the spacing between clusters or $5 \times 10^{-7} \text{ cm}$. With these assumptions, the value of D at 2,173 K is $\sim 3 \times 10^{-14} \text{ cm}^2 \text{ s}^{-1}$.

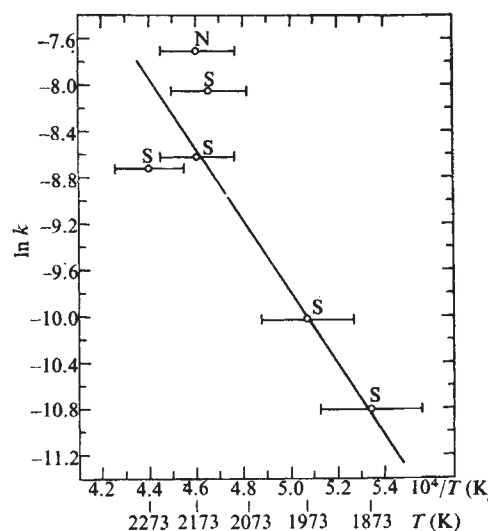


Fig. 4 A plot of $\ln k$ values against $10^4/T$ for all the experiments. The slope of the drawn line corresponds to an activation energy of 60 kcal mol⁻¹. S, synthesised diamond; N, natural diamond.

This value of the diffusivity of nitrogen in diamond may be compared to the diffusivity of carbon in diamond from data supplied by Swalin¹¹. Swalin gave values for D_0 of $11.6 \text{ cm}^2 \text{ s}^{-1}$ and Q of 143 kcal mol⁻¹ in

$$D = D_0 \exp(-Q/RT)$$

At 2,173 K this gives $D = 6 \times 10^{-14} \text{ cm}^2 \text{ s}^{-1}$. Hence, in consideration of the nearly equal sizes of the nitrogen and carbon atoms, a reasonable value for the diffusivity of nitrogen for the type IB to type IA transformation in diamond should be in the order of $10^{-14} \text{ cm}^2 \text{ s}^{-1}$ at 2,173 K provided that the cluster model for aggregate type IA diamond is correct.

Table 2 Reaction rates at different temperatures and times required to reach 50, 99 and 99.9 conversion of type IB to type IA nitrogen

T (K)	k (min ⁻¹ p.p.m. ⁻¹)	c_0 (p.p.m.)	$t_{50\%} = 1/c_0k$ (min)	Conversion times	
				$t_{99\%} = 99/c_0k$	$t_{99.9\%} = 999/c_0k$
2,273	3.2×10^{-4}	500	6.3	10 h	4.3 d
2,173	1.74×10^{-4}	500	11	19 h	8.0 d
2,073	8.9×10^{-5}	500	22	37 h	16 d
1,973	4.3×10^{-5}	500	47	77 h	32 d
1,873	1.9×10^{-5}	500	105	175 h	73 d
1,773	7.7×10^{-6}	500	260	18 d	0.5 yr
1,673	2.8×10^{-6}	500	715	49 d	1.4 yr
1,573	9.0×10^{-7}	500	2,200	150 d	4.2 yr

Initial concentration, c_0 , assumed to be 500 p.p.m. type IB nitrogen. Calculations based on: $k = A \exp(-E/RT)$ with $E = 60 \text{ kcal mol}^{-1}$ and $k = 1.74 \times 10^{-4} \text{ min}^{-1} \text{ p.p.m.}^{-1}$ at 2,173 K.

Conclusions and discussion

At temperatures between 1,873 K and 2,273 K the type IA nitrogen state in diamond is the stable form, not the type IB state. In this temperature region the diamond states responsible for both group A and group B type IA absorption bands seem to be stable. The experiments also suggest that the transformation of dispersed type IB nitrogen into type IA nitrogen is more favourable kinetically than diffusion of dispersed nitrogen out of the diamond.

The activation energy for the type IB to type IA nitrogen transformation is approximately 60 kcal mol^{-1} (2.6 eV) over the temperature range 1,873 K to 2,273 K. Assuming a cluster model for aggregate type IA nitrogen, an approximate value for the diffusivity of nitrogen for the type IB to type IA transformation should be in the order of $10^{-14} \text{ cm}^2 \text{ s}^{-1}$ at 2,173 K.

Continuing studies should give some insight into some old questions about diamonds. For example, it was believed that the platelets seen in type IA diamonds with the transmission electron microscope were composed of a nitrogen-carbon complex. New evidence suggests the platelets are composed of interstitial carbon^{5,12}, with nitrogen apparently necessary for the formation of the platelets but not being incorporated into them in any appreciable amount. Transmission electron microscope studies on the present transformed type IB synthesised diamonds should help resolve the role of nitrogen in the formation of platelets. A parallel study on X-ray diffraction spikes should confirm any findings about platelets.

It has been considered that the various optical features of type IA diamond such as the $1,280 \text{ cm}^{-1}$ absorption band or the N-3 absorption and emission system with its sharp zero phonon line were found only in natural diamonds. Hence, the finding of the N-3 emission system in the Hannay diamonds was taken as proof for their natural origin¹³. This assumption is probably correct since these features are related to aggregate nitrogen and the required annealing conditions for transforming the type IB dispersed nitrogen found in synthesised diamonds into type IA aggregate nitrogen have not been attainable until recently. These annealing conditions were almost certainly not attainable in the laboratory during Hannay's time. Present experiments have shown what these conditions are: 1,673 K at 60 kbar for ~ 50 d or 1,873 K at 60 kbar for ~ 7 d for 99% conversion of type IB to type IA nitrogen (see

Table 2). These times and temperatures are appreciably greater than those reported by Hannay¹⁴. More detailed experiments will be needed to differentiate between natural and synthesised diamonds in the future.

The colouration change that occurs on annealing type IB crystals suggests that large, yellow type IB synthesised diamonds¹⁵ that are grown slowly to obtain gem quality crystals could be turned into colourless crystals by proper annealing.

It now seems quite certain that during the growth of diamond in a nitrogen containing environment, whether naturally or in the laboratory, nitrogen deposits substitutionally in diamond in an atomically dispersed state. With prolonged dwell times in the growth environment, the nitrogen state changes, quite probably forming aggregates which impart characteristic type IA properties.

The time for completion of the reaction is rather short on a geological time scale. The fact that some natural diamonds do contain a substantial amount of atomically dispersed nitrogen suggests that these crystals were exposed to temperatures above 1,300 to 1,500 K for only relatively short times during growth and later transport to the surface through the internal churning of the mantle.

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Terrestrial lead isotopic evolution and formation time of the Earth's core

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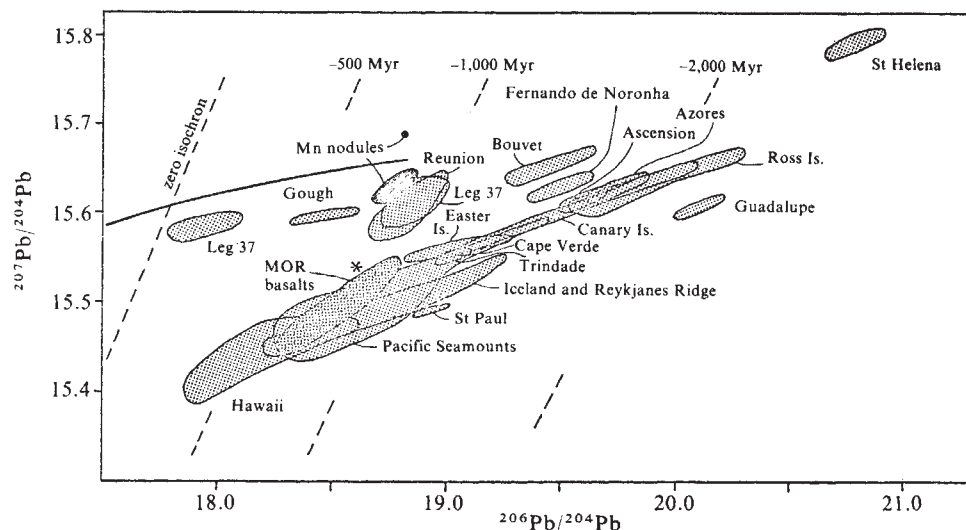
The radiogenic nature of Pb in average crust and mantle probably requires some interaction between mantle and core during core formation and is thus incompatible with heterogeneous accretion models for the Earth. Pb isotope evolution models for hot and cool accretion indicate that core formation was not quasi-simultaneous with accretion although the core was probably 95% completed within the first 700 Myr of the Earth's history.

THE high precision Pb isotope data which has become available in the past few years enables average Pb isotopic composition for the sampled mantle and crust (Fig. 1) to be estimated. The mid-oceanic-ridge (MOR) basalts occupy a restricted field, whereas oceanic islands extend linearly to more radiogenic Pb. The most reliable estimate of average crustal Pb is probably given by the present-day ratios of the conformable Pb ore growth curve¹ which represents the Pb isotopic evolution of average crustal Pb^{2,3}. Another estimate is obtained from Pb in manganese nodules and

oceanic sediments. This Pb has its origin in continental waters, as seawater has an order of magnitude lower Pb concentrations, and as the mean resident time of Pb in seawater is short ($\sim 3,500 \text{ yr}$)⁴ it will give the average Pb of the eroded continental surface. There is a good agreement between both estimates (Fig. 1). It is evident from the difference in the $^{207}\text{Pb}/^{204}\text{Pb}$ ratio that continental crust, at least in the past $1 \times 10^9 \text{ yr}$ but possibly from early on in the Earth's history, has followed a different Pb isotopic evolution than oceanic mantle. This precludes a steady growth of the continental crust until the present.

If the crust-mantle U concentration is estimated to be 0.035 p.p.m.⁵, then a μ value ($^{238}\text{U}/^{204}\text{Pb}$) of 9.5 will yield 0.0037 p.p.m. ^{204}Pb . From continental crustal abundances for U of 1.9 p.p.m. (ref. 6) and for Pb of 12.5 p.p.m. (ref. 7), a mass balance calculation gives 32% of U and 27% of Pb in the crust now. Despite uncertainties of up to 50% in some estimates a crustal μ value of 11 derived from these figures is in excellent agreement with a present-day μ value of 10.75 resulting from the Pb ore growth curve¹. Although Pb and U are both strongly enriched in the crust, U is probably only slightly enriched relative to Pb. A similar geochemi-

Fig. 1 Lead isotopic composition of oceanic basalts and manganese nodules. Solid line, conformable lead ore growth curve; *, estimate of average sampled crust-mantle Pb isotopic composition. Source of data: MOR basalts (ref. 9); oceanic islands (refs 9, 21–25); manganese nodules (refs 26, 27 and R.V. unpublished); conformable Pb ore growth curve (ref. 1); reference isochrons (refs 10, 17).



cal behaviour of U and Pb is demonstrated by only considering oceanic Pb: MOR basalts seem to be derived from a previously depleted source region. They contain low concentrations of LIL (large ion lithophile) elements and contain also less radiogenic Pb (and Sr) than oceanic islands basalts^{8,9} (Fig. 1). Enrichment in LIL elements is again correlated with a slight increase in the μ value. Because of this geochemical behaviour the best estimate for average oceanic Pb is probably not the average MOR basalt but a point shifted somewhat to the more radiogenic side within the MOR basalt field. From these figures the average Pb isotopic composition for the sampled crust-mantle system is estimated to be $^{206}\text{Pb}/^{204}\text{Pb} \approx 18.6$ and $^{207}\text{Pb}/^{204}\text{Pb} \approx 15.54$.

Comparison with meteoritic Pb (Fig. 1) reveals that the sampled mantle and crust is radiogenic in excess of a single-stage evolution (an evolution with constant U/Pb ratios) dating from 4.57×10^9 yr, when the Pb in the Earth had the same isotopic composition as the Pb in meteorites; this time is commonly regarded as the time of accretion of the Earth. This radiogenic nature is particularly pronounced when the revised U decay constants of Jaffey *et al.*¹⁰ are used, and is demonstrated by large negative single-stage model ages from -600 Myr to $-1,000$ Myr for MOR basalts and ~ -800 Myr for the estimated average sampled crust-mantle system (Fig. 1). This cannot be attributed to uncertainties in the estimated crustal Pb. For the average crust-mantle system to fall on the meteoritic zero isochron, the average crust would have to be extremely unradiogenic ($^{206}\text{Pb}/^{204}\text{Pb} \sim 14-15$, corresponding to a model age of $\sim 2,500$ myr). This would be far beyond the limits of uncertainty in this estimate, and contrary to the observation that U and Pb are on the whole very little fractionated. As it is unlikely that the Earth is substantially younger than meteorite parent bodies (a single-stage evolution, under the condition of a large U-Pb fractionation during accretion, would yield less than 4,420 Myr), it follows, therefore, that the average Pb of the sampled crust-mantle system is almost certainly radiogenic in excess of a closed system evolution. U must have been added and/or Pb been removed from this system sometime during its history since the accretion of the Earth.

It has been postulated that the unsampled (presumably lower) mantle is the source of the excess U. For example, Church and Tatsumoto⁹ suggest that mantle convection extends to depth exceeding the pyroxene-garnet transformation, resulting in a strong exclusion of large ion lithophile (LIL) elements from the mantle mineralogy and in their concentration in an interstitial phase. Separation of this interstitial phase would leave the lower mantle depleted and the upper mantle enriched in LIL elements, allowing μ to increase with time in the upper mantle. This model, however, is incompatible with a chondritic rare earth abundance pattern for the bulk crust-mantle system. As the source of MOR basalts seems to be depleted in LIL elements and thus in light rare earth elements and has been so for much of the Earth's history¹¹⁻¹³, the model proposed by Church and Tatsumoto would demand that the lower mantle, and thus the bulk crust-mantle

system be even more strongly depleted in light REE than the source of MOR basalts. This is extremely unlikely.

An obvious, and possibly the only conceivable way a radiogenic crust-mantle system may be generated would be by enrichment in U and depletion in Pb during core formation. This requires some interaction between mantle and core during core formation (but does not necessarily entail equilibrium between core and mantle) and is thus incompatible with, and a strong argument against, a heterogeneous accretion model for the Earth.

In the following, two simple models of core formation based on Pb isotope data are presented. Model I is identical with the model of Oversby and Ringwood¹⁴ who start from the same basic premise; however, the revised U decay constants of Jaffey *et al.*¹⁰ will significantly alter the results. In both models it is assumed that all U present in the undifferentiated Earth is accumulated in the mantle and none is retained in the core. This seems to be a reasonable assumption from our knowledge of the chemistry of the core¹⁵. The Pb partition coefficient between a silicate melt and iron-nickel with minor amounts of sulphide or silicate has been measured by Oversby and Ringwood¹⁴. Their best estimate for the core-mantle Pb partition coefficient is $D_{\text{Pb}}^{\text{c/m}} = 2.5 \pm 0.5$ although some uncertainty exists because of the required large extrapolation to high pressures.

Model 1

The specific boundary condition (in addition to those outlined above) for this model is: the time of core formation is assumed to be short in comparison to that required to change significantly the Pb isotopic composition by decay, so that core formation may be regarded as a discrete and instantaneous process. This approximation is valid if core formation occurred within a few Myr. This will reduce the Pb isotopic evolution to a simple two-stage evolution model with constant μ in the first stage, for which is valid¹⁶:

$$\frac{\mu^{\text{Earth}}}{\mu^{\text{m}}} = \frac{A^{\text{m}} (\exp \lambda' t_{\text{c}} - 1) - B^{\text{m}} (\exp \lambda t_{\text{c}} - 1)}{B^{\text{m}} (\exp \lambda t_{\text{p}} - \exp \lambda t_{\text{c}}) - A^{\text{m}} (\exp \lambda' t_{\text{p}} - \exp \lambda' t_{\text{c}})} \quad (1)$$

where $A^{\text{m}} = (^{206}\text{Pb}/^{204}\text{Pb})_{\text{m}} - a_{\text{p}}$, $B^{\text{m}} = [(^{207}\text{Pb}/^{204}\text{Pb})_{\text{m}} - b_{\text{p}}] / 137.88$, λ and λ' are the ^{235}U and ^{238}U decay constants, and μ is the $^{238}\text{U}/^{204}\text{Pb}$ ratio normalised for U decay to its present-day value; m and c stand for the average crust-mantle and core, respectively, and a_{p} , b_{p} are the Pb isotope ratios at t_{p} (the time of accretion of the Earth) derived from meteorite Pb¹⁷. A relation between the μ value for the bulk Earth in the first stage from t_{p} till t_{c} (μ^{Earth}) and the average μ value for the crust-mantle system (μ^{m}) in the second stage from t_{c} till t_{o} (the present) is derived through the crust-mantle U and Pb concentrations $C_{238\text{U}}$, $C_{204\text{Pb}}$ and partition coefficients $D_{\text{U}}^{\text{c/m}}$ and $D_{\text{Pb}}^{\text{c/m}}$.

$$C_{204\text{Pb}}^{\text{Earth}} = C_{204\text{Pb}}^{\text{m}} [\beta_0 D_{\text{Pb}}^{\text{c/m}} + (1 - \beta_0)] \quad (2)$$

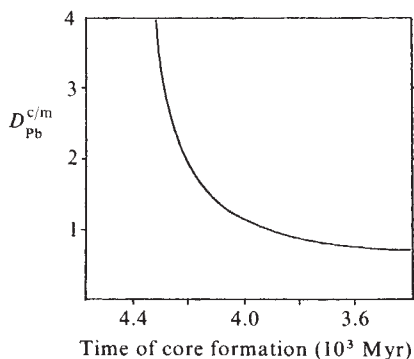


Fig. 2 Model 1: time of core formation as a function of the core-mantle Pb distribution coefficient.

and because $D_U^{c/m} = 0$

$$C_{238U_0}^{Earth} = C_{238U_0}^m (1 - \beta_0) \quad (3)$$

where β_0 is the relative mass of the core at the present ($\beta_0 = 0.32$). Equations (2) and (3) combine to

$$\mu^{Earth} = \frac{\mu^m (1 - \beta_0)}{\beta_0 D_{Pb}^{c/m} + 1 - \beta_0} \quad (4)$$

The results are plotted in Fig. 2. The sum of all uncertainties involved (except uncertainty in the distribution coefficient) may be approximated to be variations in the single-stage model age. The revised U decay constants of Jaffey *et al.*¹⁰ causes a change in the model age by a factor of 2 to 3 and, therefore, the results are significantly different from those obtained by Oversby and Ringwood¹⁴, who concluded that the time interval between accretion and formation of the core was probably less than 100 Myr and because of such a short interval, accretion must have resulted in a hot initial Earth. Assuming that the U-decay constants (which are the most important source of any uncertainty) will not significantly change with any further revision, core formation according to this model cannot have occurred before 200 Myr and probably not later than 500 Myr after accretion. Values for μ^{Earth} are strongly dependent on the assumed Pb partition coefficient: they are about 4.0, 4.8, and 6.5 for $D_{Pb}^{c/m}$ equal to 3, 2, and 1, respectively. The average μ -value for crust-mantle is about 9.4. Contrary to the interpretation given by Oversby and Ringwood¹⁴, this model is only compatible with an initially solid Earth being heated by radioactive decay until the metallic phase suddenly segregated into a core.

Model 2

If core formation is regarded as a first-order rate process, the rate of growth of the core is given by

$$\frac{d\beta}{d\tau} = \frac{-d\beta^*}{d\tau} = \varepsilon\beta^* \quad (5)$$

where β^* is fractional mass of core material, which is present at any time in the mantle either as metallic particles, droplets, or in solution, and is equilibrated with the surrounding silicate phase; ε is the growth constant for the core. The mass of core at time τ is then

$$\beta_\tau = \beta_\tau^* (\exp \varepsilon \tau - 1) \quad (6)$$

if it is assumed that at the time of accretion of the Earth the mass of core had been zero. Equation (6) is written in the commonly used geological timescale (t) where t_0 is zero and time is counted positive into the past:

$$\beta_t = \beta_0^* (\exp \varepsilon t_p - \exp \varepsilon t) \quad (7)$$

From equation (7) follows the mass of core at time t in relation to its present mass:

$$\beta_t = \frac{\beta_0 (\exp \varepsilon t_p - \exp \varepsilon t)}{\exp \varepsilon t_p - 1} \quad (8)$$

The concentration C_N of a nuclide N in the residual mantle after the n -th batch of core material, $\Delta\beta$, has segregated from it is related to the concentration of the nuclide in the mantle (relative mass: $1 - \beta$) immediately before this event by

$$C_N^m (1 - \beta)_{n-1} = [\Delta\beta D_N^{c/m} + (1 - \beta)_n] C_N^{n,m} \quad (9)$$

where $D_N^{c/m}$ is the core-mantle distribution coefficient for the nuclide N . The change in concentration during this event is given by $\Delta C_N = C_N - C_{N(n-1)}$ and the change in the relative mass ($1 - \beta$) of the mantle by $\Delta\beta = (1 - \beta)_{n-1} - (1 - \beta)_n$. The concentration at time t is therefore related to the mass of core at time t by

$$\int_{C_{Ntp}^m}^{C_{Nt}^m} \frac{-d C_N^m}{C_N^m} = (D_N^{c/m} - 1) \int_0^{\beta_t} \frac{d\beta}{1 - \beta} \quad (10)$$

which integrates to

$$C_{Nt}^m = C_{Ntp}^m (1 - \beta_t)^{D_N^{c/m} - 1} \quad (11)$$

The μ value of the mantle at t will be (approximation $D_{Pb}^{c/m} - C_{Pb}^c/C_{Pb}^m \approx C_{204Pb}^c/C_{204Pb}^m$)

$$\mu_t^m = \mu_{tp}^m (1 - \beta_t)^{-D_{Pb}^{c/m}} \quad (12)$$

Finally, the growth of radiogenic ^{206}Pb since t_p is given by

$$\left(\frac{^{206}\text{Pb}}{^{204}\text{Pb}} \right) - a_p = \int_0^{t_p} \lambda \mu_t^m \exp \lambda t \, dt \quad (13)$$

where μ_t^m is an exponential function of time as described by equations (8) and (12). A similar equation may be written for the ^{235}U - ^{207}Pb decay system.

The integrals for the $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ ratios were solved numerically. Values obtained for the core growth constant ε for core-mantle Pb partition coefficients of 5, 3, and 2 are 8.5, 5.4, and $3.5 \times 10^{-9} \text{ yr}^{-1}$, respectively. For a partition coefficient value as low as 1, ε cannot be computed. Figure 3a shows the growth of the core with time for the three partition coefficient values and in Fig. 3b is plotted the growth of μ_t^m for the same values. The calculated present-day μ value for average crust-mantle is about 9.5 and is almost independent of the distribution coefficient and growth constant. The μ value for the bulk Earth is, however, above all determined by the Pb distribution coefficient ($\mu^{Earth} = 1.38 \pm 0.05$, 3.0 ± 0.1 , 4.4 ± 0.1 for $D_{Pb}^{c/m} = 5$, 3, 2, respectively). This model is compatible with hot accretion which left the Earth completely or partially molten and enabled metallic droplets to sink towards the centre and form a continuously growing core.

Discussion and conclusions

Pb isotope data are probably only compatible with homogeneous accretion of the Earth. Simple models corresponding to the two extreme homogeneous accretion hypotheses of (1) relatively cool accretion with subsequent heating by radioactive decay and (2) hot accretion, show that core formation was probably not quasi-simultaneous with accretion, as is widely held, but occurred after or during a period of several hundred Myr following accretion. The exact definition of the time of core formation, t_c , in model 1 and mass of core, β_t , in model 2, is ultimately dependent on and determined by the rate of Pb exchange between metallic and silicate phases at the pressures and temperatures prevalent for each point in the protomantle. If Pb exchange with the surrounding silicate phase is negligibly small when segregated metallic bodies reach, for example, metre scale, then this is the time measured by t_c (model 1); subsequent growth to larger units and shifts in their relative position to the centre of the Earth will not be measured. Therefore t_c will be a maximum time for the actual core formation. Similarly, in model 2 all (in this example) metre-sized or

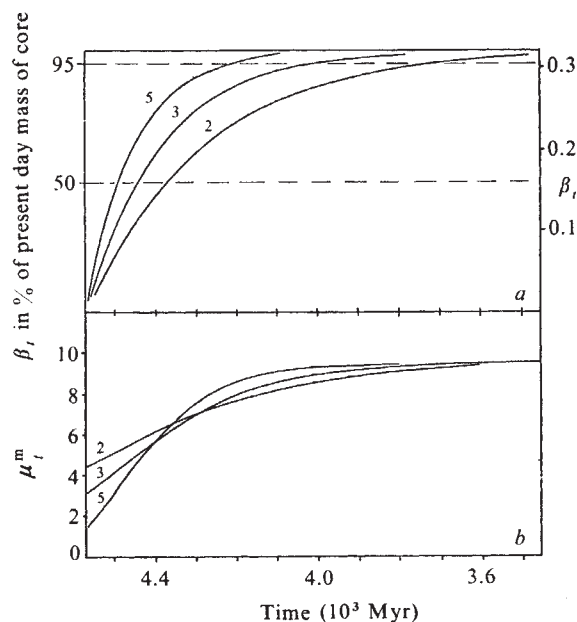


Fig. 3 Model 2: *a*, growth of relative mass of core, β_t , with time for three values of the core growth constant ϵ obtained for core-mantle partition coefficients of 5, 3, and 2. *b*, Change of the mantle μ value with time for $D_{Pb}^{c/m} = 5, 3$, and 2. The bulk Earth μ value is primarily dependent on the core-mantle Pb partition coefficient.

larger metallic bodies would be included in the quantity β_t , regardless of whether they were uniformly distributed within the protomantle, or formed a layer, or were part of the actual core.

The shortcomings of both these simple models are severe: for example, energy released during core formation and cooling only on the surface of the Earth will cause non-uniform and time-variant temperature distribution, only the latter is taken into account in model 1, though not in model 2. Non-uniform temperature distribution will mean that partial segregation of the metallic phase may occur in some parts of the Earth earlier than in others. Then the calculated time of core formation in model 1 will become an average value. In model 2 this will cause the rate of core growth ϵ to be variable in time. Therefore both models are oversimplifications which may only serve to demonstrate that there was probably a minimum time interval of some hundred Myr between accretion and completion of core-formation, but which cannot provide any detailed model of the process; such a model would have to take account of non-uniform temperature distribution.

Model 1 would entail the core being in equilibrium with the mantle for all non-radiogenic elements; from model 2 it would follow that the total core is now in disequilibrium with respect to all elements. This disequilibrium may easily be calculated from equations (2) and (11) and acts in such a way that all elements which partition into the core are depleted in the mantle below their equilibrium abundance and vice versa. It seems, however, that siderophile elements are on the contrary less depleted in the mantle

than demanded by equilibrium considerations^{18,19}. This apparently leads to a paradox. Enrichment in the mantle of elements which are expected to partition into the core seems to demonstrate disequilibrium between core and mantle, on the other hand radiogenic Pb suggests that equilibrium between core and mantle had once been reached. Assuming that this disequilibrium is real and not just an indication of our lack of knowledge of distribution coefficients at high pressures and temperatures, a solution might be that temperatures within a considerable part of the Earth have never been sufficient for a metallic phase to separate. After core formation, however, this primordial material must have been thoroughly homogenised with depleted mantle to account for the radiogenic Pb excess. It is not possible to attribute this low temperature, primordial material to a chilled outer surface: a chilled protocrust would be expected to have a low μ value and would thus be depleted in ^{207}Pb for any given ^{206}Pb value relative to the mantle. On the contrary, the continental crust is enriched in ^{207}Pb . But Ringwood's model¹⁸ of accretion and core formation would (with the exception of the time of core formation) fit these constraints. The interior of the Earth accretes cool, a metal phase separates only from the hot outer regions and collects into bodies which sink to the centre without attaining equilibrium with the material which they pass through. Subsequently, depleted and undepleted mantle regions were homogenised by convection. The ^{207}Pb excess in the continental crust may indicate that the major part of the crust was formed from depleted mantle before homogenisation was achieved.

A time interval of some hundred Myr does not invalidate the precipitation hypothesis²⁰ for the origin of the moon. Although Ringwood states²⁰ that the moon forms after separation of the Earth's core, there is no reason why this has to be so, as long as the surface temperature on the Earth during the last stages of accretion is sufficiently high to evaporate silicates but not high enough to volatilise iron¹⁸.

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Neurotransmitter synthesis and uptake by isolated sympathetic neurones in microcultures

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Assays of isolated single sympathetic neurones show that their transmitter functions can be either adrenergic or cholinergic depending on growth conditions. The data suggest that the number of transmitters made by most mature individual neurones is restricted.

DEVELOPMENT of transmitter functions by primary sympathetic neurones cultured *in vitro* is remarkably plastic¹⁻³. When grown alone in certain conditions, rat sympathetic neurones become primarily adrenergic. They develop the functions necessary for synthesis, uptake, storage, and release of catecholamines, but show relatively low levels of cholinergic function⁴⁻⁶. These



neurones can form morphological synapses on each other which contain high concentrations of small granular vesicles⁷⁻⁹ but show no physiological activity, probably because the neurones are not very sensitive to catecholamines¹⁰. Cultures of these same neurones, grown with large numbers of non-neuronal cells, however, develop many cholinergic properties. They synthesise acetylcholine (ACh) and form functional cholinergic synapses with each other, heart, and skeletal muscle^{1-3,11-15}.

Medium 'conditioned' by incubation for 2 d with appropriate non-neuronal rat cells can also induce ACh synthesis and cholinergic synapse formation^{5,9,16,17}. The extent of ACh synthesis and cholinergic synapse formation increases as the neurones are exposed to higher concentrations of conditioned medium^{9,16,17}. In contrast, development of the functions necessary for catecholamine synthesis and accumulation is repressed in a dose-dependent fashion by increasing concentrations of conditioned medium¹⁶. Among the neurones that establish themselves in culture there is little cell death and different doses of conditioned medium do not significantly change the number of neurones that survive^{16,18}. Therefore, it seems likely that a single population of immature sympathetic neurones is able to differentiate in more than one direction. By examining single cells, we hoped to determine how the development of transmitter function is regulated in individual neurones. In particular, is the population shifted from primarily single adrenergic neurones to primarily single cholinergic neurones by growth in high concentrations of conditioned medium? In the intermediate conditions do most single neurones simultaneously express both cholinergic and adrenergic functions? Our biochemical studies on these questions have been reported in preliminary form¹⁹. Motivated by similar objectives, Furchspan *et al.*¹² and Landis⁸ have recently made physiological and morphological observations on individual neurones grown in conditions similar to ours.

Synthesis of transmitter

When primary sympathetic neurones were plated at low density into micro-wells grown in conditioned medium lacking the cholinergic 'factor', and examined after 4 weeks, many wells were found to lack neurones. Others contained one visible neurone (Fig. 1), while still others contained 2-20 neurones. If only single neurones were plated initially, a Poisson distribution of neurones per well could be anticipated. In fact, small clusters of neurones were also plated so a typical plate contained 35 empty wells, 2-5 wells with single neurones, and 20 wells with more than one neurone.

Fig. 1 An isolated sympathetic neurone. This montage shows an isolated neurone, grown on a dried collagen drop, with its neuritic ramifications. To assay transmitter synthesis the neurones were grown in Falcon 3034 Terasaki culture plates. One of these plates contains 60 wells with a capacity of 10 μ l each. Approximately 50 μ g of rat tail collagen in 10 μ l 0.1% acetic acid²⁰ was evaporated in each well. The plates were then sterilised under an ultraviolet lamp. Sympathetic neurones were dissociated from newborn rats (Charles River CD)^{4,21}. In some experiments, these were plated at appropriate dilutions directly into the micro-wells and allowed to settle for 2 h before conditioned medium (CM) or control L-15 CO₂ medium with methocel, rat serum, and nerve growth factor flooded the wells^{4,16}. In other experiments, cardiac cells were dissociated from the hearts of newborn rats with collagenase (EC 4.24.3) (Worthington type 1, 1 mg ml⁻¹ in Ca²⁺, Mg²⁺-free Hanks solution for 20 min at 37 °C) and approximately 500 cells were seeded in each well. After 2 h, the wells were flooded with complete L-15 CO₂ medium containing rat serum, but lacking methocel and nerve growth factor. After 1-4 d, the heart cells had formed monolayers in the wells. At this point, the wells were seeded with neurones at an appropriate dilution and incubated with complete L-15 CO₂ medium^{4,16} containing methocel, rat serum and nerve growth factor. In all our cultures, proliferation of heart and other non-neuronal cells was prevented by γ -irradiation (5,000 rad over 25 s) 2 d after the neurones were seeded. Neurones adhering to the sides of these wells were scraped off 2 d after plating with a tapered Teflon rod. Cells were grown for 28-35 d in the same L-15 CO₂ medium, which was changed on day 2 and every 4 d thereafter. The wells were then scanned with the phase contrast optics and only those in which the neurones could be counted easily were used for studies of transmitter synthesis and uptake. In preparation for labelling, the plates were washed twice with complete L-15 CO₂ medium lacking methocel and three times with complete L-15 CO₂ medium lacking methocel, tyrosine, and choline. The neurones were starved for 30 min in this medium and the plates washed three times more with complete L-15 medium lacking methocel, tyrosine and choline. At this point 10 μ l of this same medium, containing 0.25 μ g freshly added ascorbate, 700 pmol ³H-L-tyrosine (10-20 Ci mol⁻¹), and 700 pmol ³H-choline (10.1 Ci mmol⁻¹), were added to each well. At 4 and 8 h later, an additional 1 μ l of the same medium, containing 0.25 μ g fresh ascorbate, was added to each well. After 12 h incubation at 37 °C, the plates were washed three times more with the same medium, without isotope. The wells were drained, and each well was filled with 10 μ l of pH 1.9 buffer containing unlabelled standards^{4,22}. The plates were stored for 1-48 h at -20 °C and wells were thawed and frozen twice before the contents were applied to paper, and the transmitters were separated by high voltage electrophoresis (6,000 V, 90 min)²². The sections of paper containing dopamine, NA and ACh were identified²², cut out, and counted⁴. The counting efficiency (~25%) was determined by drying on paper known quantities of the tritiated precursors. CM containing the cholinergic 'factor' was obtained by incubating 20 ml of complete L-15 CO₂ medium for 2 d with a 75-cm² monolayer of the rat glioma cell line C6 (ATCC CCL107²³) in a Falcon 7004 tissue cultured flask. CM lacking the 'factor' was obtained by incubating the same amount of medium with a monolayer of embryonic chicken heart cells in the same type of flask¹⁶. The chicken heart cells were obtained from 10-d chicken embryos by collagenase dissociation, using the procedure used for rat hearts. Single neurones are apparently more fastidious than mass neuronal cultures, so that standard culture conditions used in these studies were not always successful. It was particularly difficult to obtain firm adhesion of single neurones to collagen sheets. All manipulations had to be performed very gently, and it was useful to verify that the neurone remained in the well at the end of the many medium changes that followed the incubation with labelled precursors. Since 1 fmol of labelled transmitter provided only 10-20 c.p.m., it was important to clean the electrophoresis tanks before each run and to use standards, blotting paper, scissors, and scintillation fluid that had not been contaminated by other experiments. With these precautions, the background was reduced to approximately 30 c.p.m. in favourable experiments. Unknown causes gave higher backgrounds in some experiments and these results were discarded. All vials were counted twice for 10 min and the results were averaged. The pieces of paper containing transmitters were compared to equal sized paper pieces, cut just in front and behind the transmitter spots.

Most of these wells were incubated with ³H-tyrosine and ³H-choline for 12 h and synthesis of ³H-ACh and ³H-noradrenaline (NA) was measured after the incubation. The results of a typical experiment are presented in Fig. 2. Significant amounts of ³H-ACh were not detected in any well. Synthesis of ³H-NA was not detected in any wells lacking visible neurones. In the 10 wells containing a single neurone, however, 10-50 fmol of NA synthesis was seen. Wells containing more neurones made, on the average, more ³H-NA. (Only the results from the wells containing 0, 1 and 4 and 5 neurones are presented in Fig. 2 because the other classes provided no additional insight.) These results show that single neurones

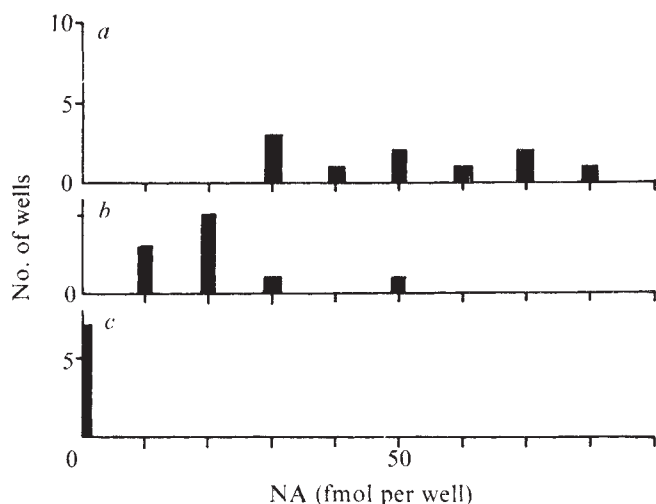


Fig. 2 ^3H -ACh and ^3H -NA synthesis by neurones grown in control medium. The neurones were grown for 29 d in complete L-15 CO_2 medium supplemented with 20% of the same medium first conditioned on a monolayer of chicken embryo heart cells. The neurones in each well were counted and incubated with ^3H -choline and ^3H -tyrosine. The results are plotted in separate graphs for those wells containing: a, 0; b, 1, or c, 4 or 5 neurones. No synthesis of detectable ^3H -ACh was seen in any well. The amount of ^3H -NA synthesised rounded to the nearest multiple of 10 fmol, is plotted on the abscissa. The number of wells making a particular amount of ^3H -NA is plotted on the ordinate.

make detectable quantities of ^3H -NA, but not ^3H -ACh, when grown in these conditions. Every neurone synthesised and accumulated detectable transmitter, but the amount made by a single cell may differ by at least a factor of 5.

Why do single neurones make such differing quantities of ^3H -NA? To see whether differences in transmitter synthesis simply reflected differences in metabolic activity of neurones, we measured the acid precipitable radioactivity in ^3H -tyrosine and ^3H -choline derivatives. The results are shown in Fig. 3. There is a correlation, but not a strong one between the amounts of radioactivity in the acid precipitable and NA fractions. Some neurones were metabolically active, but produced relatively little transmitter. By this criterion, the neurones seem to have differentiated to different extents after culture for 4 weeks *in vitro*. Immature neurones have been seen in superior cervical ganglia as late as 3 weeks after birth²⁴. Our results could reflect this documented *in vivo* heterogeneity, but alternatively, may reflect limitations in our methods for nerve culture.

The primary sympathetic neurones can also be grown in conditions which promote cholinergic development. Figure 4 shows the results of one representative experiment in which the neurones were plated onto monolayers of rat embryo heart cells and labelled 32 d later with ^3H -tyrosine and ^3H -choline. Wells containing heart cells but not neurones made neither ^3H -NA nor ^3H -ACh. In the wells containing neurones, the average synthesis of ^3H -ACh was seven times that of ^3H -NA, but single neurones did not synthesise both transmitters in this ratio. In 26 of the 30 wells that seemed to contain a single neurone we detected synthesis of

8–53 fmol of ^3H -ACh without detectable ^3H -NA synthesis. In three wells there was synthesis of 9–17 fmol of ^3H -NA, but no detectable ^3H -ACh. In only one single neurone well was there synthesis of both ^3H -ACh and ^3H -NA, 6 fmol and 3 fmol respectively. The results suggest that there are at least two and possibly three classes of neurones, differing in their patterns of transmitter synthesis. Support for this interpretation is provided by the data obtained from wells containing more than one neurone. Synthesis of ^3H -ACh was seen in all five wells containing two neurones, but ^3H -NA was seen in only one, suggesting that four wells contained two cholinergic neurones while one well may have contained one adrenergic and one cholinergic neurone. In the wells containing more neurones, highly disparate ratios of ^3H -ACh to ^3H -NA synthesis were seen. These fluctuations also suggest that more than one class of neurones developed in these wells.

Several experiments have been done on single cells grown in conditions where the average ratio of ^3H -NA synthesis is closer to one. In these experiments, the neurones developed in the presence of L-15 CO_2 medium conditioned by previous growth with the rat glioma cell line C6. Neuronal mass culture grown in this medium make significant amounts of ^3H -ACh¹⁶. The results with the single neurones are summarised in Table 1. In 50% conditioned medium approximately 45% of the neurones made detectable levels of ^3H -ACh, but not ^3H -NA; roughly the same percentage made ^3H -NA, but not ^3H -ACh. In about 5% of the wells synthesis of both transmitters was seen. In 20% conditioned medium, 90% of the neurones were adrenergic; the remainder were cholinergic. The

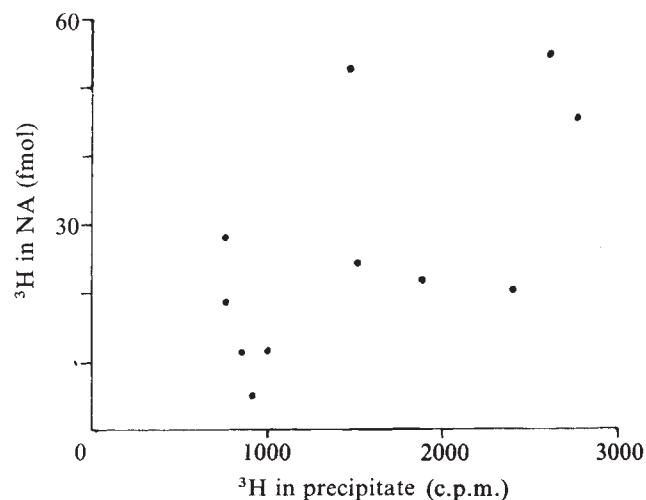


Fig. 3 Incorporation of radioactivity into the acid-precipitable and NA fractions of single neurones. The cells from Fig. 1 were used for this experiment. After high voltage electrophoresis, strips of paper including the regions within 1 cm of the origin were placed on a Millipore filter with the sides to which radioactivity had been applied uppermost. These strips were washed with cold 10% trichloroacetic acid, and counted in a scintillation counter to determine the amount of acid precipitable radioactivity. The background from five wells containing no neurones averaged 42 c.p.m. and was subtracted. c.p.m. acid precipitable radioactivity is plotted on the ordinate; fmol ^3H -NA in the precipitate on the abscissa.

Table 1 Transmitter synthesis by single neurones grown in various conditions

Growth conditions	ACh neurones (a)	NA neurones (b)	ACh and NA neurones (c)	Negative neurones (d)	ACh:NA ratio
Control	0	18	0	0	<0.02
20% CM	2	21	0	0	0.15
50% CM	15	15	2	2	2.4
Heart cells	26	3	1	0	7.0

Pooled results are given of all experiments on neurones grown in control medium, in 20% and 50% conditioned medium (CM), and on rat heart monolayers. Procedures for growing neurones in control medium and on rat heart monolayers were as described in Figs 1 and 3. Neurones grown with 20% or 50% conditioned medium (conditioned with C6 cells) were labelled 25–28 d after plating. The values are the numbers of single neurones making only ACh (a), the number making only NA (b), the number making ACh and NA (c), the number making neither transmitter (d), and the average ratio of ACh to NA synthesis in the pooled results, including wells with more than one neurone.

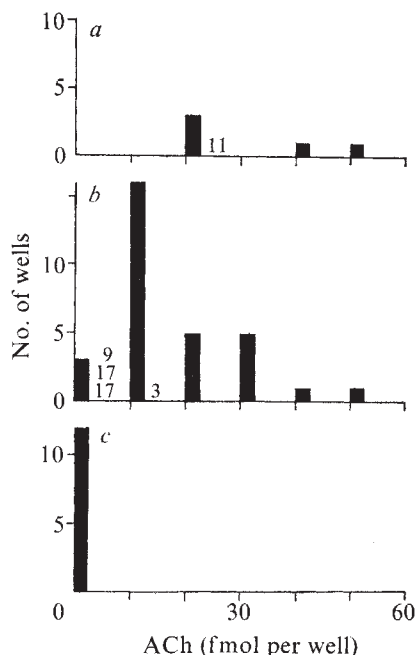


Fig. 4 ^3H -ACh and ^3H -NA synthesis by neurones grown on heart cells. Culture age was 32 d. The neurones in each well were first counted, then the wells were washed and incubated with ^3H -choline and ^3H -NA (see Fig. 1 legend). The results are summarised in separate graphs for wells containing 0 (c), 1 (b), or 2 (a) neurones. The fmol ^3H -ACh synthesised by a particular well is plotted on the abscissa. The number of wells in which there was a particular amount of ^3H -ACh synthesis is plotted on the ordinate. Values for ^3H -ACh synthesis are rounded to the nearest multiple of 10 fmol. (There were no examples, though, in which 2–5 fmol ^3H -ACh was synthesised, and only one example in which a cholinergic neurone made less than 8 fmol ^3H -ACh. That neurone seemed to make 6 fmol ^3H -ACh and 3 fmol ^3H -NA). When there was detectable ^3H -NA synthesis in a well, the amount has been written directly above or to the right of the appropriate bar. Averaged, ^3H -ACh synthesis by all these neurones was seven times as high as ^3H -NA synthesis.

pooled results of all our experiments are summarised in Table 1. It seems that most cells become primarily cholinergic or adrenergic, but that the percentage committed to either path depends on the growth conditions.

Uptake of noradrenaline

Transmitter synthesis is only one parameter of neuronal function. Many neurones also have high affinity uptake systems for the transmitters that they synthesise, store, and release (compare ref. 25). We have found that sympathetic neurones cultured in conditions which promote adrenergic development possess such an uptake system for NA, with a K_m of $\sim 1 \mu\text{M}$, which is blocked by cocaine. Storage of this NA is abolished by reserpine⁵. Mass cultures of sympathetic neurones grown on monolayers of rat heart retained the ability to concentrate and store ^3H -NA, but at a reduced rate (data not shown). One possibility was that no ^3H -NA uptake occurred in neurones making ACh.

To see whether cholinergic neurones could also concentrate and store NA, single neurones were grown on rat heart monolayers for 30 d and were then labelled with ^3H -choline, and ^3H -NA (50 nM). The results are presented in Fig. 5. Wells containing no neurones neither made ^3H -ACh nor concentrated ^3H -NA. Significant ^3H -NA uptake was seen in all 57 wells containing a single neurone; ^3H -ACh synthesis was seen in 53 of these same wells. Clearly, most neurones have an uptake system for NA. The few that did not do both may have been cells, like those in Fig. 4, that synthesised only ^3H -NA and not ^3H -ACh.

Conclusion

Examination of transmitter synthesis and uptake functions in developing sympathetic neurones has revealed the existence of two or three different classes of cells. The first class, virtually the only

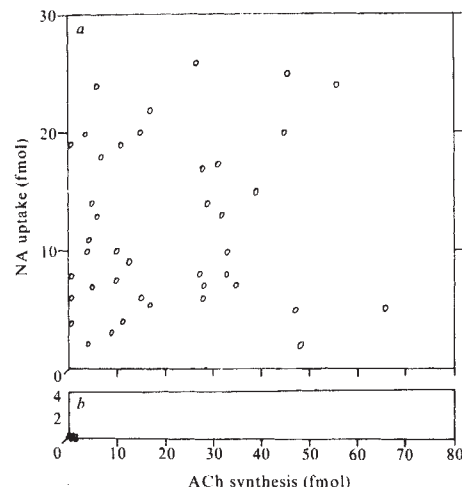
class present when the neurones are grown alone, is adrenergic. The individual neurones synthesise considerable NA, but no detectable ACh. In mass culture, they have a high affinity uptake system for NA^{5,6}. They almost certainly correspond to the neurones described by Furshpan *et al.* and Landis^{1,2,8} that, when stimulated, evoked only adrenergic responses in heart cells and have varicosities filled with small granular vesicles after KMnO_4 fixation to localise endogenous NA.

The second class synthesises considerable amounts of ACh, but no detectable NA; however, these neurones retain the ability to concentrate and store exogenous NA (Fig. 5). These probably correspond to the neurones described by Furshpan *et al.*¹² and Landis⁸ which contained no endogenous small granular vesicles, formed cholinergic synapses on themselves and evoked cholinergic but not adrenergic responses in heart muscle cells.

Furshpan *et al.*¹² and Landis⁸ have also presented convincing evidence for the presence in young cultures of a third class of neurones that releases both cholinergic and adrenergic transmitters on heart muscle cells. Their experiments did not show that single cells synthesised both transmitters; conceivably they could have concentrated NA made by other neurones on the dish. They also did not show whether the neurones were transiently or permanently bifunctional. It is possible that all young neurones responding to the cholinergic inducer substance(s) might pass through a bifunctional phase. Our experiments with more mature neurones do not contribute to the study of these very interesting cells. Although we have found occasional neurones that seemed to synthesise both NA and ACh (Fig. 4, Table 1), they were rare enough to be possible artefacts. Either a hidden neurone or terminals from one whose cell body had been lost might explain our results. Neuronal numbers can be counted with high, but not absolute, reliability with phase contrast optics. This limitation in our methods makes it difficult to study rare classes of neurones. In the future, of course, neurones shown electrophysiologically to be releasing both ACh and NA can also be assayed for ACh and NA synthesis from labelled precursors.

The two major classes, those neurones exhibiting cholinergic and adrenergic biosynthetic activities, are present in different proportions in different growth conditions. In our experiments, the fraction of neurones committed to ACh synthesis increased as the ratio of ACh to NA synthesis in the whole population increased (Table 1). In addition to the shift in the population of neurones caused by conditioned medium or heart cells, it is also possible that these 'cholinergic' factor(s) when present in higher concentrations,

Fig. 5 ^3H -NA uptake and ^3H -ACh synthesis by the same neurones. Neurones were grown for 30 d in complete L-15 CO_2 on monolayers of embryonic rat heart cells. The neurones in each well were counted. Then they were incubated with ^3H -choline and unlabelled tyrosine for 10 h. Each well then received $2 \mu\text{l}$ of the same medium containing 50 fmol ^3H -NA (22 Ci mmol^{-1} , NEN). The final ^3H -NA concentration was 50 nM. Wells were washed and cells collected 2 h later. The results are for wells containing 0 (b) or 1 (a) neurone. The fmol ^3H -ACh synthesised is on the abscissa; the fmol ^3H -NA uptake is on the ordinate. Each circle represents a single well.



might cause neurones committed to the cholinergic decision to produce higher levels of ACh. The heterogeneity in the individual neuronal responses in our experiments precluded a firm answer on this point.

Virtually every single neurone that survived made at least one transmitter in our experiments. This result suggests that there are no large populations of 'silent' neurones making neither ACh nor NA in mass cultures grown in adrenergic or cholinergic conditions. Since the same number of neurones survive in cultures grown in several different concentrations of cholinergic 'factor'¹⁶, selective death of adrenergic cells in cholinergic conditions and the reverse are unlikely to explain the alterations in transmitter synthesis. Instead, individual neurones probably retain developmental plasticity at the time they are plated on the dish.

Most primary sympathetic neurones did not make detectable amounts of ACh and/or NA (Figs 2,4; Table 1), suggesting that regulatory mechanisms limit transmitter synthesis by single sympathetic neurones. Such mechanisms may force individual cells to respond in a 'flip-flop' fashion to the cholinergic 'factor'. The young dual functional neurones described by Furshpan *et al.*¹² may have been in a transitional state when they were examined. This may also have been true for the few neurones that we observed which made two transmitters. The heterogeneity in response of the individual neurones to a given level of the cholinergic 'factor' may reflect the heterogeneity in developmental time course that has been documented *in vivo*²⁴, or it could reflect microheterogeneity in the tissue culture environment. Either could explain the broad distribution in rate of transmitter synthesis by single neurones.

In some cells, however, regulation of transmitter synthesis is more complex than predicted by the 'flip-flop' model. In several systems there is evidence for the simultaneous presence of more than one transmitter in a single neurone²⁶. Neuroblastoma clones have been isolated which contain high levels of tyrosine hydroxylase and choline acetyltransferase²⁷. Similarly, the rat pheochromocytoma PC12 synthesises both NA and ACh²⁸⁻³⁰. Some, but not all sub-clones of PC12, retain high levels of both tyrosine hydroxylase and choline acetyltransferase. While single cell analysis has not been done on this clonal line, the available results make it unlikely that cell lines are forced to respond in a 'flip-flop' fashion. Possibly, the clonal lines are frozen in an embryonic state of development. Alternatively, choices between transmitters are not obligatory, but are highly probable in the conditions in which the cultured sympathetic neurones develop.

The uptake of NA by neurones that synthesise ACh is consistent with the observation that NA can be concentrated into small granular vesicles of identified single neurones that secrete ACh⁸. Experiments on mass cultures had indicated that NA uptake was reduced 10-20-fold in cholinergic growth conditions (data not shown), so we had expected NA uptake to be restricted to adrenergic cells. Possibly the proteins required for the uptake and storage of NA are regulated independently of the NA biosynthetic

enzymes. Indeed, neuroblastoma clones that have one, but not another of the biosynthetic enzymes for NA have been described³¹, suggesting that every adrenergic function can be independently regulated. Alternatively, all the sympathetic neurones may develop some adrenergic functions before they respond to the cholinergic 'factor'. In support of this interpretation, sympathetic cells in newborn rats are known to be weakly fluorescent²⁴ and to initially form *in vitro* endings with high concentrations of small granular vesicles which are lost with cholinergic development³. At least one sympathetic neurone-like rat pheochromocytoma clone also synthesises significant amounts of NA in the 'undifferentiated' state²⁸. Possibly, then, the proteins required for synthesis, uptake, storage, and release of NA are made in most young sympathetic neurones. Their synthesis is reduced or eliminated by contact with the cholinergic 'factor', but the activities which are more stable persist in lower amounts in the developing cholinergic cell.

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Detection of acceptor sites on human lymphocytes for antigen-specific T cell factors

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Mouse antigen-specific T cell factors are absorbed by human peripheral blood lymphocytes at acceptor sites. The acceptors are products of HLA-linked genes, which may be human immune response genes.

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THE immune response or Ir genes control the levels of immune responses to specific antigens. In many species, an important group of such genes has been shown to lie in the major histocompatibility complex (MHC) (ref. 1). Considerable interest attaches to the Ir genes of man, in particular because of the associations between antigens of the HLA system (the human MHC) and certain diseases, many of an autoimmune character, such as ankylosing spondylitis, Addison's disease, juvenile diabetes, coeliac disease and multiple sclerosis^{2,3}. Ir gene polymorphism is

thought to play an important part in these disease associations. Some progress has been made in demonstrating human Ir genes, particularly in certain allergies⁴⁻⁶, but a major obstacle in experimental work is the ethical and practical problem of immunising volunteers and their families with antigens of scientific interest. In order to avoid these difficulties, we have attempted to detect products of Ir genes on human lymphocytes *in vitro*, using methods which have been successful in the mouse. In particular we have looked for Ir gene products involved in immunological cell interactions. Two types of such products have been described recently, namely the antigen-specific T cell factors, which are soluble mediators of T cell-B cell⁷ and T cell-T cell⁸ interaction, and the cell-bound acceptors, the sites on the lymphocyte surface where reaction with the T cell factors takes place⁷. The factors include antigen-specific helper and suppressor products which are assayed by their abilities to replace the helper or suppressor effects of T cells *in vivo* or *in vitro*⁹⁻¹², while the acceptors are defined by the capacity of lymphocytes to absorb the specific factors⁷. In the mouse, both the factors and the acceptors are coded at least partially by genes in the I region of the H-2 complex^{7,13,14}, and it has been proposed that they are the structural products of the Ir genes which are found in the same region. Thus, the inherited absence of either the helper factor for a particular antigen or its acceptor, is associated with low response to that antigen in mice⁷. In this report we describe a remarkable feature of the mouse specific helper factors, namely their ability to be absorbed by human peripheral blood lymphocytes (PBL). On interaction with the mouse factor, human PBL are activated and proceed to antibody production *in vitro*¹⁵. This suggests that critical mechanisms for the interaction between helper and acceptor structures have been preserved in speciation. Furthermore, the acceptor sites of human PBL seem to be polymorphic: for antigens such as synthetic polypeptides under murine Ir gene control, lymphocytes of different human individuals can be shown to be either high or low absorbers of the relevant T cell factors. Family analyses indicate that the acceptor genes are linked to the HLA complex, and preliminary mapping of two loci has been achieved in HLA-recombinant families. These findings provide an approach to the detection and mapping of the Ir genes of man.

T-cell factor interaction with human lymphocytes

Antigen-specific T cell helper factors were prepared against sheep erythrocytes (SRBC) and the synthetic polypeptides (T,G)-A-L (batch 1383) and (Phe,G)-A-L (batch 223). The method, detailed elsewhere^{7,9}, involved *in vitro* incubation of specifically educated (primed) mouse (C57B1/6) thymocytes together with antigen for 6-8 h. The cell-free supernatants of the educated T-cell cultures were the source of T-cell factors. Factor activity was assayed, before and after absorption by human lymphocytes by transfer into irradiated (700 rad) syngeneic mouse recipients together with bone marrow cells and antigen^{7,9}. Plaque forming cells (PFC) against the relevant antigen were measured 8-10 d later for SRBC or 12-14 d later for polypeptides, in the spleens of the animals, using appropriately coated erythrocytes⁹ and compared with suitable controls. Donors of human lymphocytes were employees of Hoffman-La Roche, Basel, and members of their families. Samples of 20 ml of blood were taken in heparin and lymphocytes separated by Ficoll sedimentation¹⁶. The cells were washed three times in medium free of calcium and magnesium and maintained before use in phosphate buffered saline (PBS) containing 10% foetal calf serum. For absorption of T-cell factor, 10⁷ PBL from each donor were sedimented and resuspended in 2-3 spleen equivalents of T-cell factor (approximately 0.5 ml of the supernatant of educated T-cell culture), without further addition of antigen. After incubation for 30 min in ice, the lymphocytes were removed by centrifugation and the absorbed supernatants were mixed with mouse bone marrow cells and antigen and transferred into irradiated recipients (five per group) as described above. All the absorption tests on human lymphocytes were carried out strictly blind, with the identity or HLA type of individuals concerned being revealed only after final results had been obtained. HLA-typing was by standard techniques, using the facilities of the Basel Institute for Immunology.

Table 1 PFC responses before and after absorption of specific mouse T cell factors with lymphocytes of different human donors

PBL donor (initials)	Mouse factor specific for		SRBC
	(T,G)-A-L	(Phe,G)-A-L	
-(Controls)	2,100 ± 280	3,600 ± 312	4,400 ± 360
GE	10* ± 8	25* ± 15	71* ± 35
GM	1,700† ± 210	15* ± 11	40* ± 26
HB	15* ± 11	2,700† ± 188	55* ± 26
RF	2,400† ± 235	2,850† ± 296	112* ± 65

Results as geometric means of PFC per spleen ± s.d. (five mice per group).

*High absorber.

†Low absorber.

Incubation of the antigen-specific mouse helper factors with human PBL results, in many cases, in the complete removal of factor activity from the supernatant. This phenomenon is consistently observed in randomly-selected human donors for the helper factor specific for SRBC. Table 1 shows a typical absorption result, in which 10⁷ PBL of four donors all removed the SRBC-specific factor. No failure to absorb this factor has yet been observed. But, when the mouse factors specific for the synthetic polypeptides (T,G)-A-L and (Phe,G)-A-L were used, the PBL of some but not all donors were found to absorb. In the majority of cases, clear-cut distinction between high and low absorbers was possible. Table 1 illustrates this—PBL of donors GE and HB absorbed the (T,G)-A-L specific factor, whereas those of GM and RF failed to remove the factor. A similar result is seen for the (Phe,G)-A-L factor, where this time GE and GM are high absorbers, and HB and RF are low absorbers. It thus seems that acceptor sites for the (T,G)-A-L and (Phe,G)-A-L specific factors are expressed on the PBL of some but not all individuals, while the ability to absorb SRBC-specific factor has been found on PBL of all individuals so far tested. Moreover, as Table 1 shows, all combinations of high and low absorption seem to be possible in different individuals (with the exception of low to SRBC factor).

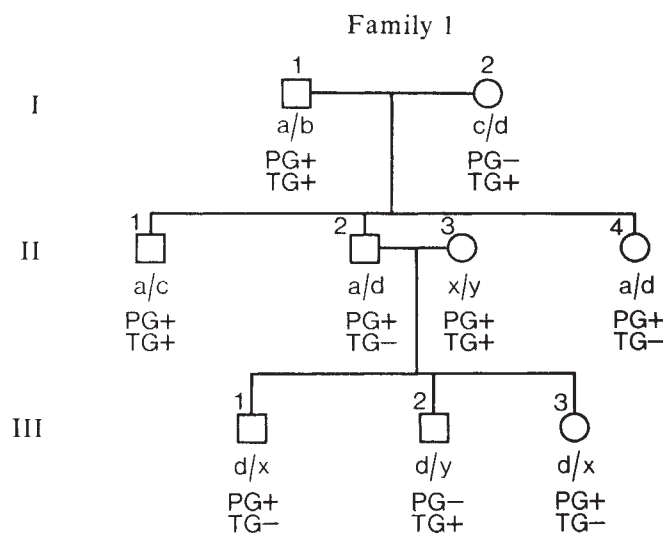
These findings have so far been extended to 41 random individuals, and the results are summarised in Table 2. Approximately 34% of the individuals tested are low absorbers of the (T,G)-A-L factor, 27% are low absorbers of the (Phe,G)-A-L factor, and 15% are low absorbers for both, although it should be noted that a smaller number of donors have so far been tested for (Phe,G)-A-L (26 tested) than for (T,G)-A-L (41 tested). These findings closely resemble those made in the mouse, where the B cells of some low responder strains fail to absorb the (T,G)-A-L or (Phe,G)-A-L specific factors and various combinations of the low absorption phenotypes for the different factors have been found. Note that in the mouse, lack of the acceptor site for an antigen-specific T cell factor can be correlated with low response of the animal to the antigen⁷. The absorption characteristic of each individual was a stable trait which could be detected very reproducibly, as indicated by perfect concordance in a re-test of 25 donors carried out blind, 6 months after first testing.

Family analysis strongly suggests that the abilities to absorb the T-cell factors for the two synthetic polypeptides are indeed inherited as simple Mendelian traits. A typical pedigree in three generations is shown in Fig. 1. This family illustrates the dominance of the high absorption characteristic. For example, the

Table 2 Absorption of specific mouse T cell factors by human lymphocytes: summary of phenotypes observed

No. of phenotypes	(T,G)-A-L	Factor absorption (Phe,G)-A-L	SRBC
13	High	High	High
3	High	Low	High
11	High	NT	High
6	Low	High	High
4	Low	Low	High
4	Low	NT	High

Approximate gene frequencies: (T,G)-A-L, high 0.4, low 0.6; (Phe,G)-A-L, high 0.5, low 0.5 (see discussion). NT, not tested.



	HLA haplotypes			Absorption loci	
	A	B	C	<i>p</i>	<i>t</i>
(a)	w30	12	w2	+	-
(b)	w24	w35	w4	(?)	+
(c)	w23	w17	-	-	+
(d)	w32	w40	w3	-	-
(x)	1	8	-	+	-
(y)	29	12	-	-	+

Fig. 1 Pedigree of abilities to absorb mouse T cell factors. PG+ and PG-, respectively the high and low absorption phenotypes for the (Phe,G)-A-L-specific mouse T cell factor. TG+ and TG-, respectively the high and low absorption phenotypes for the (T,G)-A-L-specific mouse T-cell factor. a, b, c, d, x and y, represent the HLA HLA haplotypes of the family members. *p* and *t*, the genetic loci controlling respectively the absorption of the (Phe,G)-A-L and (T,G)-A-L factors. With respect to the *p* and *t* loci, the signs + and - denote high and low absorption alleles respectively. The key shows the deduced linkage phase of *p* and *t* with each haplotype.

mating II.2 $\times$ II.3 between two PG+ individuals gives both PG+ and PG- offspring; this can be interpreted as an intercross between two heterozygotes of genotype p^+/p^- , in which the 'high absorber' gene p^+ behaves as dominant over its p^- allele. A similar interpretation applies to the inheritance of the TG character in the offspring of the mating I.1 $\times$ I.2. Thus, family data support a model in which the absorption abilities are controlled by two loci (*t*, *p*) each with two alleles (t^+ , t^- ; p^+ , p^-), high being dominant over low. Accepting this model and assuming that the population presented in Table 2 is at Hardy-Weinberg equilibrium, the gene frequencies are: $t^+ \approx 0.4$, $t^- \approx 0.6$; $p^+ \approx 0.5$, $p^- \approx 0.5$.

Acceptor relationship to HLA system

On the basis of the situation in the mouse it was obvious to look for a linkage with the HLA region, and indeed inspection of the family in Fig. 1 shows that the genes controlling the TG and PG phenotypes, assigned independently of any knowledge of the HLA typing, segregate with the HLA haplotypes. So far, no cross-over between HLA and the acceptor loci has been observed in over 20 potentially informative offspring of backcross type matings, (other than in families with known recombinations within the HLA complex as described below). Thus, close linkage between the acceptor loci *t* and *p*, and HLA is indicated, though a significant recombination fraction cannot be excluded. In this and other

families several other genetic markers were studied, either with no information (Kk, Kp, Js, Wr^a, ADA, AK, Inv) or with evidence of independent segregation (ABO, MNSs, P, Rh, Fy, Lu, Kl, Le, Xg^a, Hp, ACP₁, PGM₁, GPT, Gc, Pt and Gm).

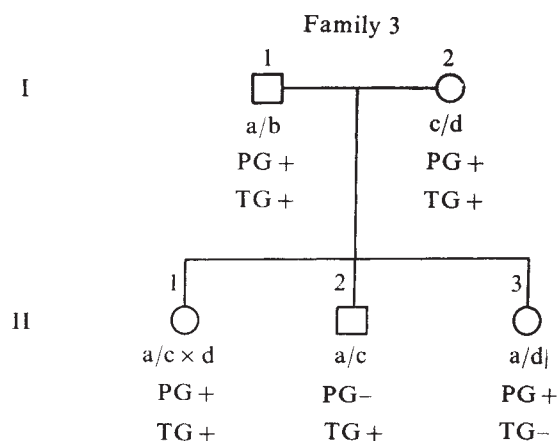
Confirmation that there are indeed two acceptor loci in the HLA complex comes from HLA-recombinant families, one of which is shown in Fig. 2. The recombinational event occurred between the HLA-A and HLA-C loci in the mother and the recombinant haplotype (denoted $c \times d$) is carried by a daughter (II.1). This pedigree shows that crossing over must also have occurred between the acceptor loci *t* and *p*, and that the acceptor locus *t* must be on the HLA-A side of the cross-over event, while the acceptor locus *p* is on the HLA-C side. Figure 3 illustrates this in skeleton form. Further recombinant families are being studied to localise the acceptor genes further.

In some instances, for HLA antigens of the A, B, C series a trait under study is associated with a given marker at another locus of the HLA region, that is, the two traits are in linkage disequilibrium¹⁷. Analysis of over 40 individuals rules out such a possibility for the TG and PG phenotypes in regard to HLA (ABC), even with the two frequent supertypic specificities w4 and w6, which split the HLA-B antigens into two main groups¹⁸. It could be more informative to study the associations with the D polymorphism (mixed leukocyte reaction) and Da (D-locus associated) antigens, which are the equivalent of Ia antigens in the mouse.

Discussion

Our results show that human peripheral blood lymphocytes carry acceptor sites for the mouse T cell factors for the synthetic polypeptides (T,G)-A-L and (Phe,G)-A-L. The abilities of being a 'high absorber' or a 'low absorber' are individual stable characters, which are inherited as simple Mendelian traits linked to the HLA

Fig. 2 Absorption of mouse T-cell factors in a pedigree containing a recombination in the HLA complex. Abbreviations as in Fig. 1. a, b, c, and d represent the HLA haplotypes of the family members, $c \times d$ being the recombinant haplotype.



	HLA haplotypes			Absorption loci	
	B	C	A	<i>p</i>	<i>t</i>
(a)	8	-	1	-	-
(b)	7	-	w24	+	+
(c)	w15	w3	3	-	+
(d)	7	-	2	+	-
(c $\times$ d)	7	-	3	+	+

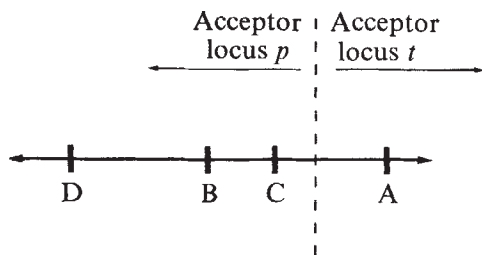


Fig. 3 HLA complex indicating preliminary mapping of the two acceptor loci *p* and *t*. *p*, acceptor locus for (Phe,G)-A-L factor, *t*, acceptor locus for (T,G)-A-L factor.

complex. This situation closely resembles that found in the mouse, where acceptor genes have been demonstrated in the I-region of H-2 and are good candidates for the Ir genes of that species. We therefore propose as a working hypothesis that the acceptor sites detected on human lymphocytes by these methods are products of the Ir genes of man and that high and low absorptions are equivalent to high or low immune responsiveness. To support this proposition, we must test the responsiveness of each individual to the antigen in question. Culture conditions have recently been established for the development of antibody responses by human lymphocytes, in which human PBL are stimulated with antigen and mouse T cell factor *in vitro*^{14,15}. Thus, the correlation of factor absorption with responsiveness can now be tested.

The results described with HLA-recombinant families indicate the existence of at least two acceptor loci separable by crossing over. In the mouse, the acceptors are I-region products and carry the Ia antigenic determinants found predominantly on B lymphocytes⁷. In man, at least two loci in HLA have been shown to code for Ia-like B cell alloantigens, one associated with or identical to the HLA-D locus while the other is associated with the HLA-A locus¹⁹. A similar situation seems to exist in the Rhesus monkey²⁰. There are also two loci determining the antigens stimulating the mixed lymphocyte reaction, another property of Ia antigens, with similar mapping^{21,22}. In addition, disease associations suggest that some Ir genes may be closely linked to the HLA-D locus³. Therefore, on the basis of the detection of lymphocyte acceptor

sites described here, we propose the existence of two Ir gene loci (or regions) in the HLA complex and suggest that one of these is likely to be associated with HLA-D and the other with HLA-A. Further mapping studies are in progress to test this model. The existence of two Ir gene regions in the HLA complex could provide the basis for a selective advantage of certain haplotypes and hence lead to the linkage disequilibrium which is a feature of the distribution of HLA alleles in the population.

These *in vitro* tests of absorption and response may make it possible to study in detail the human Ir genes, avoiding the problems of *in vivo* immunisations of human volunteers with specific antigens of scientific interest. Moreover, the use of antigens and corresponding T factors of direct medical interest, could lead to assessment of predisposition to the HLA-associated diseases (for example, multiple sclerosis, gluten enteropathy), with a higher efficiency than by the present indirect Ir typing through the HLA (A B C D) markers.

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letters to nature

Temporal and spectral variation of Cyg X-1

AMONG galactic X-ray sources, Cyg X-1 is distinctive because of its marked variability. Its X-ray intensity curve exhibits apparently aperiodic variation of large amplitudes¹⁻³. It is tempting to suppose that this peculiarity of Cyg X-1 is related to the physical properties of the accretion disk formed around the black hole presumed to accompany the supergiant HDE226868 optically identified with Cyg X-1. We report here certain features of the subsecond variability concluded from a recent rocket observation.

The rocket observation of Cyg X-1 was carried out, using a rocket launched from Kagoshima Space Center, for ~250 s starting at 0502 UT 24 September, 1975 when the source was in the low-state. The total effective area of four Xe-CO₂ filled proportional counters was 680 cm². The counts in every 0.625 ms time bin in three energy bands, nominally 1.5-5, 5-10 and 10-25 keV, hereafter referred to as L, M and H band respectively were telemetered. The counting-rate is corrected

for the small jittering of the rocket attitude.

The observed X-ray intensity profile is shown in Fig. 1 as a histogram of counts per time bin with a bin width of 0.32 s as an example. The intensity decreased by about 20% during the period of observation and in the first half period flare-like variations are more pronounced compared to the second half period.

The hardness ratio or spectral hardness averaged over 1.28 s, defined as the ratio of counting-rates of the ≤ 10 keV (L+M) and > 10 keV (H) bands, is also shown. It is clear that the spectral hardness also fluctuates in a complex manner with weak correlation to intensity variations, if there is any. The spectral variation becomes even more pronounced for shorter integration times of the order of 0.1 s. Correlation among counting-rate profiles of three energy bands corresponding to the spectral variation was investigated. It should be noted that the H-component occasionally fluctuates with characteristic time scales of the order of 0.1-1 s in a different

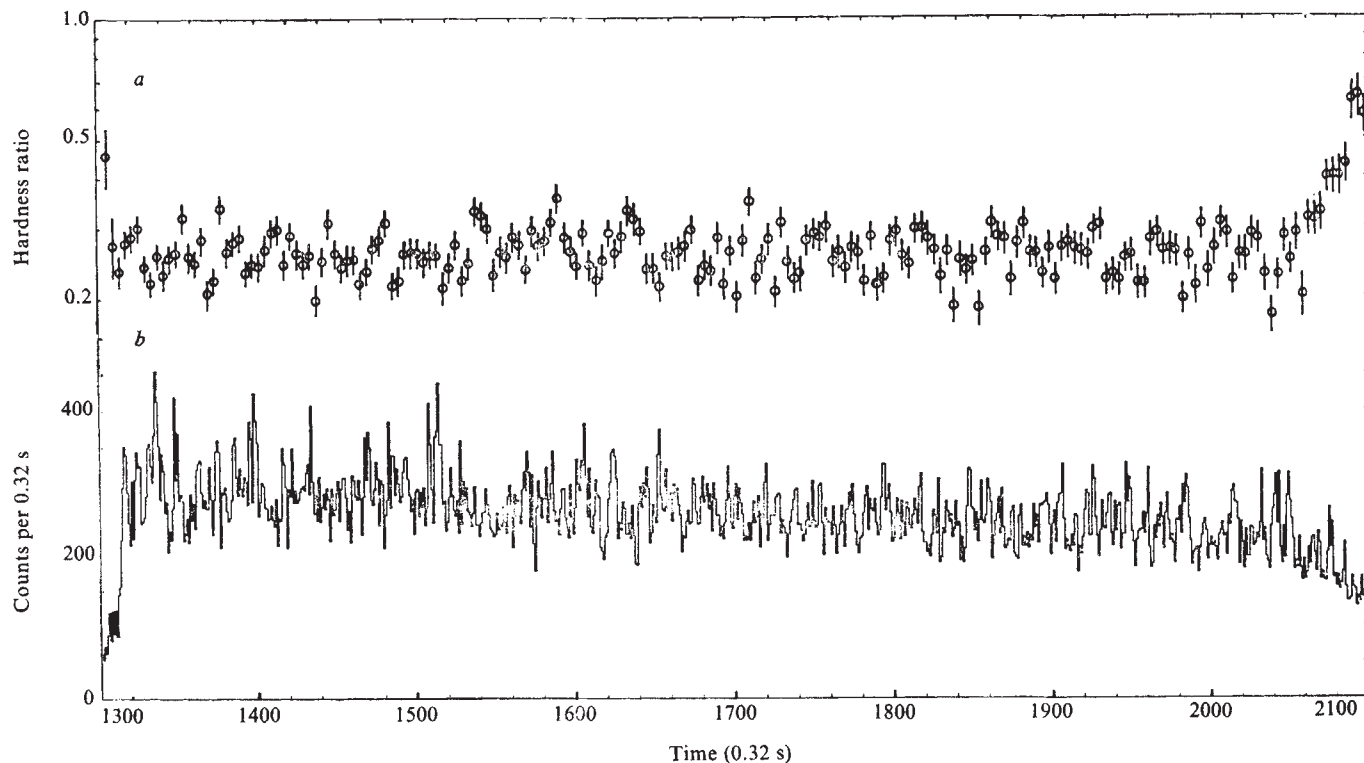


Fig. 1 Intensity and spectral time profiles corrected for aspect are shown from the acquisition of the source until the re-entry of the rocket into the atmosphere. Time resolutions for the intensity and spectral hardness ratio are 0.32 s and 1.28 s respectively. In the first half of the observation, the intensity is approximately 20% higher and the flare-like activity is stronger compared to the latter half. The time scale is shown in units of 0.32 s. *a*, 10–25 keV/1.5–10 keV; *b*, 1.5–25 keV.

fashion from the L- and M-components resulting in the spectral variation.

We define the variability coefficient, $\eta(t_b)$, as the square root of the variance, $V(t_b)_{\text{cyg}}$ due to intrinsic variation of the source, normalised to the average intensity, $x(t_b)_{\text{cyg}}$, where t_b is a bin width;

$$\eta(t_b) = \frac{(V(t_b)_{\text{cyg}})^{1/2}}{x(t_b)_{\text{cyg}}} \quad (1)$$

Statistical estimate of $\eta(t_b)$ is

$$\eta(t_b) = \frac{(V(t_b) - x(t_b))^{1/2}}{x(t_b) - y(t_b)} \quad (2)$$

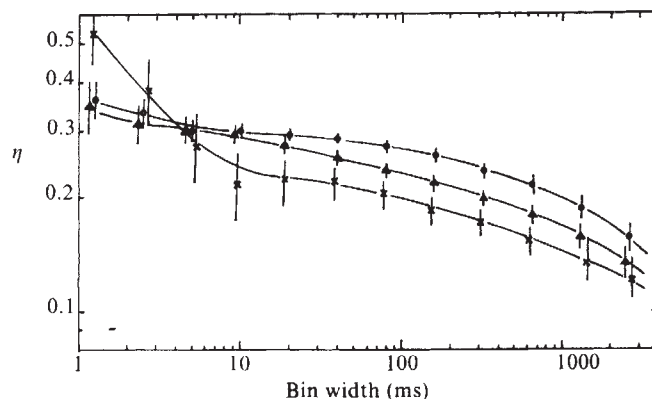
where $V(t_b)$ is the total variance, $x(t_b)$ is the average counts per bin and $y(t_b)$ is the average background counts per bin; $\eta(t_b)$, which decreases monotonically with the increase of t_b , should exhibit a significant slope over a time range corresponding to the characteristic time scale involved in the variability. Figure 2 shows the variability coefficients as a function of the bin width for respective energy bands. The curves indicate the presence of a characteristic time scale from 0.1 to 1 s for all bands and, in addition, a time scale $\lesssim 10$ ms for the H band. The 0.1–1 s time scale is also prominent in the autocorrelation function produced for this observation. The autocorrelation function is similar to that for observations already reported^{2,4}. The presence of the ~ 10 ms characteristic time scale was also notified by Canizares and Oda⁵.

In addition to the general variability, Cyg X-1 occasionally exhibits sporadic flares or bursts more than twice the average intensity and of the time scale of 1 s or a fraction thereof. The

sporadic flares from 0.1 to 1 s duration in general seem to have internal spectral structures, average spectral hardness being similar to the overall hardness: spectral softening in the flare is suggested.

It was demonstrated by Terrell⁶ that the intensity time profile and the autocorrelation function of Cyg X-1 may be reproduced by the superposition of a number of random pulses of basically constant size and decay time in analogy to the shot-noise. The idea is that the variability is caused by the statistical fluctuation of the rate of underlying unit shot. If we take the shot-noise model with impulsive shots of the decay time $\tau/2$ with the average shot rate $\lambda \text{ s}^{-1}$, the η against t_b curve approaches a constant $f/(\lambda\tau)^{1/2}$ for $\tau \leq t_b$ and tends

Fig. 2 The variability coefficients against integration time for three energy bands are shown. A characteristic time scale of the order of a second or less is indicated. A ≤ 10 ms time scale is noted for the H-band. ●, h (1.5–5 keV); ▲, M (5–10 keV); XH (10–25 keV).



to decrease asymptotically as $f/(\lambda t_b)^{1/2}$ for $\tau \leq t_b$, where f is the fraction of the shot-noise component. Thus, from Fig. 2 we estimate $\tau/2$ as 0.3 s and λ as 20 s^{-1} , if $f = 1$, for the average intensity of the source at the low-state. In Fig. 2 the variance over the time range of 0.1–1 s is shown to be smaller for higher energies (H band) suggesting that λ is larger or f is smaller compared to the low energy band.

There are several features of the variability which have to be carefully investigated to see whether they resemble a simple shot-noise model with shot pulses of a constant decay time and size. First, the spectral variation shown by the time profile of the hardness ratio in Fig. 1 cannot be created by a single kind of the shot unless we assume an involved spectral and temporal internal structure of the shot. Second, to explain the difference of η for different energy bands instead of a single kind of shots we have to introduce an artificial ensemble of shots with the variety of spectral hardness: hard shots should be more abundant and smaller in amplitude to reconcile the above results, if $f = 1$ as generally assumed, and τ is constant. If we assume $f < 1$ as suggested by Sutherland *et al.*⁷, the shot-noise model based on the constant shot can be used. If this were the case smaller f has to be assigned for higher energies, and a harder spectrum for the steady component compared to the shot-noise component and a negative correlation between the intensity and the hardness ratio are expected: the negative correlation, however, is not clear from this data. Third, the prominent flares can not be accounted for using the shot-noise model. In this experiment the estimated shot rate is about 20 s^{-1} , if $f = 1$, and the model predicts a rapidly decreasing number of the flares with increasing amplitudes. In contrast, the number of the large flares observed is much more than predicted at least for the case of $f = 1$. For the case of $f < 1$, more complex arguments are necessary concerning the shapes of the large flares and the shots. On one occasion in October 1976 large flares were observed which could not be accounted for by the superposition of the shots regardless of assumed value of f (ref. 5).

While the shot-noise model is convenient to express the variability features in terms of the parameters and is also an attractive model providing physical implications as for the turbulent nature of the accretion disk, further studies seem necessary before the basic concept of the shot-noise model is firmly established.

Figure 2 indicates the presence of an additional characteristic time scale of $\lesssim 10 \text{ ms}$ for the H component. This is interpreted in terms of the existence of significant pulses of this time scale which will be discussed elsewhere.

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Temperature measurement of interplanetary-interstellar hydrogen

PHOTOMETRIC observations can be used to measure the velocity of the Solar System through an interstellar medium and also provide an accurate method of measuring the temperature of that medium, provided that a very narrow filter is used in order to determine the emission linewidth. We present here the results obtained using a hydrogen absorption cell in conjunction with a Lyman- α photometer contained in the Soviet scientific spacecraft Prognoz-5 which was launched in November 1976. We use only the La results to measure the temperature of the interplanetary-interstellar hydrogen. The high result we obtained indicates that the Solar System may be moving through an intercloud medium heated by cosmic- or soft X-rays.

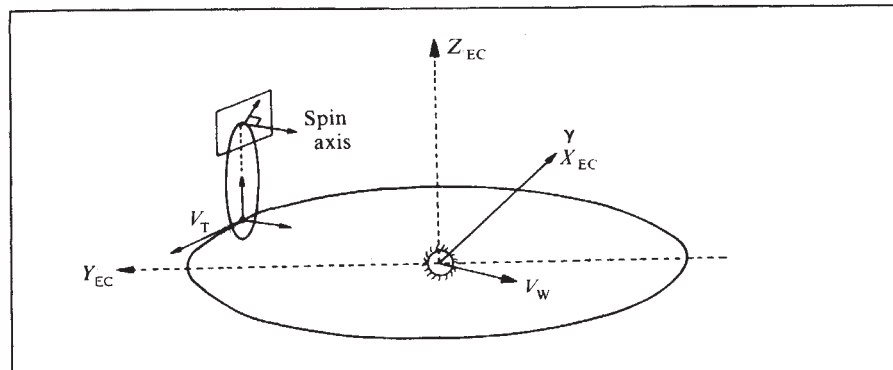
The motion of the Solar System relative to the nearby stars is characterised by a velocity vector V of 20 kms^{-1} in the Apex direction, $\alpha = 271^\circ$ and $\delta = 30^\circ$. In this motion, the Solar System is swept by neutral matter of the local interstellar medium. In particular, the flow of interstellar hydrogen atoms was first observed^{1,2} through resonance scattering of solar La photons (121.6 nm). Helium atoms were also later observed³ (at 58.4 nm). These observations confirmed the prediction that hydrogen and helium atoms can penetrate deeply into the solar system, because the heliosphere boundary is presumably quite transparent to neutral matter. From the photometric observations at 121.6 and 58.4 nm, it was found that the Solar System motion through the interstellar medium is characterised by a vector V_w , pointing to a direction $\alpha = 252^\circ$, $\delta = -15^\circ$, differing from the Apex direction by $\approx 50^\circ$. The density⁴ of hydrogen was measured in the range 0.08–0.12 atom per cm^3 while for helium³ it was found to be in the range 0.009–0.024 atom per cm^3 . An estimate of the temperature can also be derived from photometric observation but with considerable uncertainty: 10^3 – $15 \times 10^3 \text{ K}$ from La photometric observations^{4,5}, 2.5×10^3 – 10^4 K from helium observations³.

A much more accurate method to determine the temperature is to measure the line width of solar La scattered by hydrogen atoms. One attempt was made with the help of *Copernicus* high resolution spectrometer⁶, with a result lying in the range of 5 – $20 \times 10^3 \text{ K}$, rather wide because of the poor photometric sensitivity. Another measurement was obtained⁷ from a hydrogen absorption cell placed on board the Soviet probe Mars 7. Though the temperature range was narrowed to 6 – $13 \times 10^3 \text{ K}$, it was still rather large, because of poor knowledge of the direction of sight. The favoured value was $12 \times 10^3 \text{ K}$.

Results obtained recently with the same technique (a hydrogen absorption cell associated with a La photometer) yielded a much more accurate measurement of $8.8 \pm 1 \times 10^3 \text{ K}$ for the temperature of the nearby interstellar medium. The scientific Soviet spacecraft Prognoz 5 was launched on 25 November, 1976 on a very eccentric orbit, with an apogee distance to the centre of the Earth of $203 \times 10^3 \text{ km}$, and a period of nearly 4d. The spin axis of the spacecraft was pointed toward the sun, and the spin rate was 3° per s. One of the instruments placed on board was a four channel ultraviolet photometer for the study of hydrogen and helium in the interplanetary medium and in the exosphere, through the observation of resonance scattering of solar light at 121.6 nm (H, La), 58.4 nm (He), and 30.4 nm (He⁺). The La channel looked at 90° from the spin axis, with a field of view of $1.1 \times 2.7^\circ$ and a 1-s integration time of photon pulses. An absorption cell was placed in front of the photomultiplier.

The apogee of the spacecraft as observed from Earth was nearly in the direction of the North Ecliptic Pole (Fig. 1). The La intensity, recorded around the apogee and measured in the plane of rotation of the spacecraft as a function of roll angle ϕ , is shown on Fig. 2. The Earth and its hydrogen geocorona is

Fig. 1 Configuration of orbit of Prognoz-5 and its spin axis in an ecliptic coordinates system. On 1 December 1976, the Earth was nearly opposite to the direction of V_w , the velocity of the sun relative to the nearby interstellar medium.



clearly seen from $\phi = 0^\circ$ to $\phi = 170^\circ$, whereas the interplanetary L α emission is observed from $\phi = 170^\circ$ to $\phi = 360^\circ$, free of any geocoronal contamination.

The absorption cell can be electrically activated, with an optical thickness $\tau = 8$ and a temperature $T_c = 300$ K. Thus all of the emission within $\pm 1.4 \times 10^{-3}$ nm of the L α wavelength $\lambda_0 = 121.566$ nm is blocked, and the measured intensity is decreased by a factor R , the reduction factor. The absorption of the hydrogen cell is large on the geocorona (R is small) because the geocoronal linewidth is small, whereas the absorption is small (R is large) on the interplanetary background.

For a pure gaussian emission profile, the reduction factor is only a function of the temperature of emission, T and of the Doppler shift velocity V_D between the instrument and the emitting medium. This function can be accurately computed⁷ when the hydrogen cell parameters, τ and T_c are known. At apogee, the spacecraft velocity with respect to the Earth is negligible, whereas the Earth's velocity V_T on its orbit around the Sun is 30 km s^{-1} (Fig. 1); therefore there is a strong modulation of Doppler shift V_D as a function of direction of sight, defined by the roll angle ϕ , the origin of which lies in the ecliptic plane (Fig. 2). We interpret the strong dip of R around $\phi_D = 260^\circ$ as a consequence of this modulation.

The observations were made on 1 December 1976, when the spacecraft was practically 'downwind'; therefore the modulation of Doppler shift is essentially caused by V_T and not by V_w whose projection on observation plane should be small. We

assume that the velocity distribution of hydrogen atoms is not perturbed by the sun; therefore at each point of the solar system the emission profile is a pure Doppler profile characterised by T . The motion of the spacecraft through the interplanetary matter is $V_{\text{rel}} = V_T + V_w$. When the direction of sight is perpendicular to V_{rel} , the Doppler shift is zero and the temperature, T , is directly derived from the measured value of R . This should occur twice along the great circle of observation, and at these points, R should be minimum. One point is identified at the angle $\phi_D = 260^\circ$ where the minimum value R_{min} is measured, the other is somewhat occulted by the geocorona. In Fig. 3 all the measurements of R recorded for 2 d

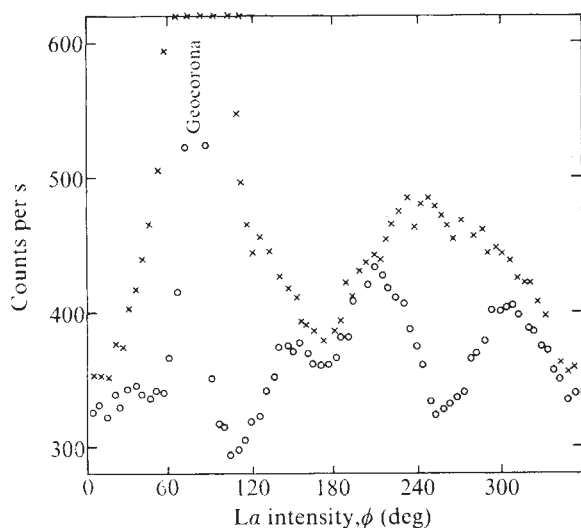


Fig. 2 The L α intensities are plotted as a function of roll angle, ϕ with and without the hydrogen-cell activated. $\times$, Cell off; $\circ$, cell on. The ecliptic plane lies at $\phi = 0$ and $\phi = 180^\circ$. The most intense part of the geocorona is not shown. The interplanetary background is clearly seen from $\phi = 180$ to $\phi = 360^\circ$, opposite to the geocorona. The absorption cell is most efficient at $\phi_D \approx 260^\circ$, where the Doppler shift is zero.

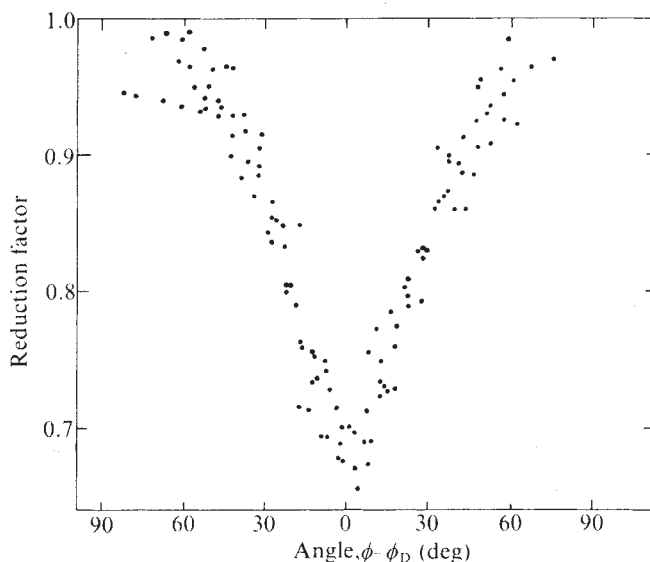


Fig. 3 All the measurements of the reduction factor, R , collected during two days are plotted as a function of $\phi - \phi_D$. A minimum value of $R = 0.68 \pm 0.01$ is deduced from this graph, yielding the temperature of the nearby interstellar medium, $T = 8,800 \pm 1,000$ K.

in the region around $\phi_D = 260^\circ$ were plotted, yielding a measured value of $R_{\text{min}} = 0.68 \pm 0.01$ from which is derived directly the temperature $T = 8.8 \pm 1 \times 10^3$ K (taking into account an uncertainty of 10% on τ). This determination, though in the range $6\text{--}13 \times 10^3$ K found with the same technique with Mars 7 is somewhat lower than the favoured value 12×10^3 K. But the photometric quality of the present experiment is so much better that we favour this value of $T = 8.8 \pm 1 \times 10^3$ K.

Such a high temperature of the nearby interstellar medium, associated with the low density of ≈ 0.1 atom per cm^3 , strongly suggest that the Solar System is moving through the so-called

'intercloud medium' (ICM) and confirm the validity of a hot ICM (ref. 8), heated by low-energy cosmic rays or by soft X rays^{9,10}.

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Absence of detectable X rays from supercluster candidate 4U0134-11

A CORRELATION between a small subset of high galactic latitude X-ray sources and superclusters of galaxies has been suggested¹. The existence of superclusters (that is, clusters of clusters of galaxies) was first discussed in the context of optical observations^{2,3}. A hot intrasupercluster gas could lead to detectable X-ray emission as well as provide a large fraction of the mass necessary to close the universe⁴. The Goddard Space Flight Center Cosmic X-ray Spectroscopy experiment onboard Orbiting Solar Observatory 8 (OSO 8) scanned directly over the position of 4U0134-11, one of the three 4U sources associated with a supercluster. We did not observe X rays from this source with an upper limit to the source strength a factor of 6 below the catalogued Fourth Uhuru (4U) strength.

Figure 1 represents the path of the OSO 8 spin axis through the region of 4U0134-11. The observation was performed between 7 and 13 July 1976. Catalogued X-ray sources in this region and their error boxes are shown. The detector is a xenon-filled proportional counter, efficient from

Fig. 1 The OSO 8 spin axis scan path during the observation of 4U0134-11. The known X-ray sources and their error boxes are shown. Portions of the scan path used to determine the background rate extend out of the picture.

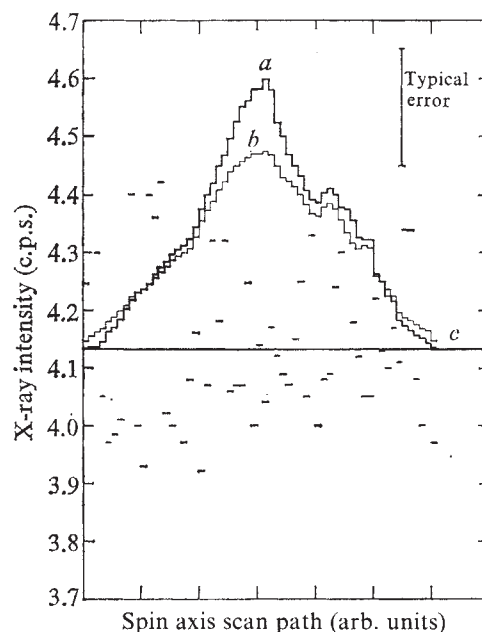
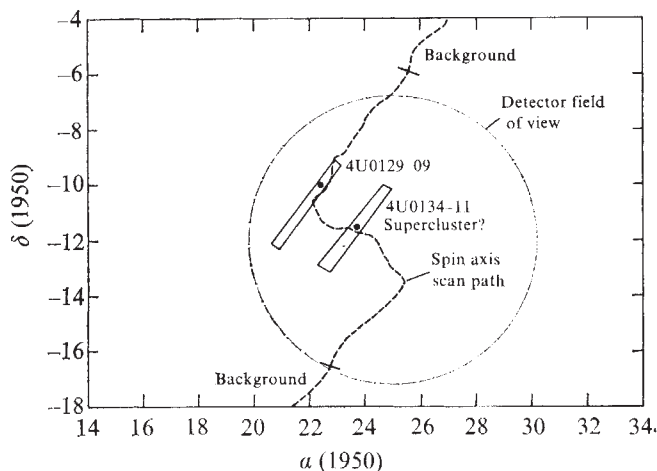


Fig. 2 Models of the X-ray intensity during the scan path superimposed on the data. Reduced χ^2 are *a*, point source 5.1; *b*, diffuse source, 4.2; *c*, no source, 1.3.

2 to 60 keV, with an effective area of 237 cm² (ref. 5). It is pointed along the spin axis and has a 5° FWHM field of view. The background rate was deduced from the rates at source-free adjacent regions.

The counting rates along the scan path were best fit by a model with a constant X-ray background and no sources. The data (and typical error) are shown in Fig. 2. The three histograms superimposed on the data are: (1) a model with 4U0134-11 as a point source at 2.1 Uhuru counts, the lower statistical limit of its 4U strength; (2) a model with 4U0134-11 as a 2° in radius diffuse source with the same strength; and (3) a model without sources. For each model the deduced X-ray background is added to the count rate and to obtain an upper limit for the strength of 4U0134-11, the strength of 4U0134-09 is set equal to zero. The reduced χ^2 for the models are 5.1, 4.2, and 1.3 respectively. These results are not sensitive to the absolute level of the background. That is, the no-source model is always by far the best fit. We obtain an upper limit at 99% confidence of 0.4 Uhuru counts for an extended (2° in radius) source at the position of 4U0134-11 (4U value = 2.7 ± 0.6 counts). Because of our large field of view and the motion of our spin axis directly over the source positions, we are not sensitive to positional changes of the sources within their 4U error boxes. Our upper limit at 99% confidence for the sum of the intensities of 4U0129-09 and 4U0134-11 is also 0.4 Uhuru counts.

The X-ray spectrum associated with the supercluster candidates is consistent with a thermal bremsstrahlung spectrum with temperature of more than 10⁸ K (ref. 6). Therefore, the fact that the Uhuru detectors⁷ are efficient to slightly lower X-ray energies than this OSO 8 detector (1.7 keV rather than 2 keV) does not affect our relative sensitivity to this hard spectrum.

Three high galactic latitude X-ray sources have been identified with superclusters^{4,6}. Our observations indicate that one of these, 4U0134-11, is either variable or spurious. Other observations⁸ with this detector indicate that it can detect X-ray sources with strengths less than the nominal 4U strength for this object and determine their spectra with comparable observing time. Variability of the X rays from 4U0134-11 on a time scale of $\sim$ years, implying a source

diameter of ~ 1 pc, would rule out a supercluster identification. We have not yet performed observations of the other 4U supercluster candidates.

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Is the Sun a pulsar?

WHILE investigating intensity variations of spectrum-lines of solar origin, periods which seem to describe the Sun's oscillation have been found. The observed period of 2.65 h is very close to 2.78 h, the predicted radial mode fundamental oscillation of the homogeneous model of the Sun. Thus some observations of the solar disk seem to confirm the existence of a radial mode pulsation, while others give no significant indication of such a period. External effects may not, however, have been filtered satisfactorily in all cases. Thus we tried to find an effect of magnetospheric origin that can be related in some way to the described observations. We report here observations of pulsations with period of 2 h 40 min.

Fluctuations of solar origin having a stable period have been noted independently by various observatories^{1,2}. From their measurements a characteristic period of 2 h 40 min can be assigned to fluctuations of the central parts of the solar disk. It was established that this event is independent of local time and shows no correlation to the starting time of observation. It is thought that the phenomenon is not due to the apparatus, but originates from the Sun. The observed oscillations can be interpreted as the Sun's pulsations, or perhaps alien effects originating perhaps from the magnetosphere and atmosphere.

The magnetosphere of the Earth can be regarded as a very sensitive measuring probe in the solar wind, which senses every perturbation of solar origin. Thus we investigated variations of the Earth's magnetic field from the viewpoint of effects caused by pulsations of solar origin. There are several magnetic recording systems in the Tihany Observatory^{3,4}. We used their data to select several periods to investigate pulsations within the range of periods indicated above.

Magnetically quiet cycles were not considered due to the relative height of local noise level. Records of great magnetic storms were useless as the variations are of large amplitudes and suppress other effects. Hence, we investigated magnetically active days with magnetic variations between 50 and 100 nT.

Diurnal sections from the data arrays of such days were individually analysed. This allowed us to eliminate phase

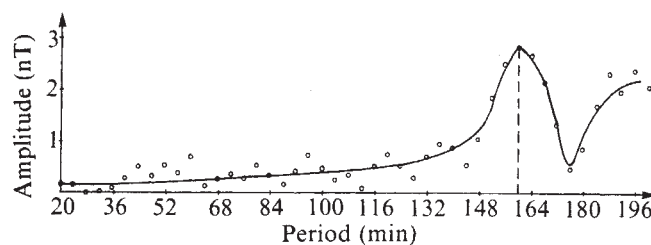


Fig. 1 Amplitude spectrum of the variations of the geomagnetic field for 10 November 1975 in the range of periods from 20 to 200 min.

spings which affected the analysis. Thus an amplitude spectrum characteristic of the diurnal part of the selected magnetically active days was obtained as shown in Fig. 1. An amplitude peak is seen to rise by a maximum of several nT out of the average noise level in the environment of the period 2 h 40 min. Most of the investigated days gave a similar result. In Fig. 2 is shown the mean spectrum for 9–11 November 1975. On several occasions no significant rising amplitude peak was found on the noise level background.

These results can be interpreted as follows. We assume that the period of 2 h 40 min is of solar origin, during magnetically active cycles the magnetosphere of the Earth and the magnetic lines of force of the interplanetary field intertwine.

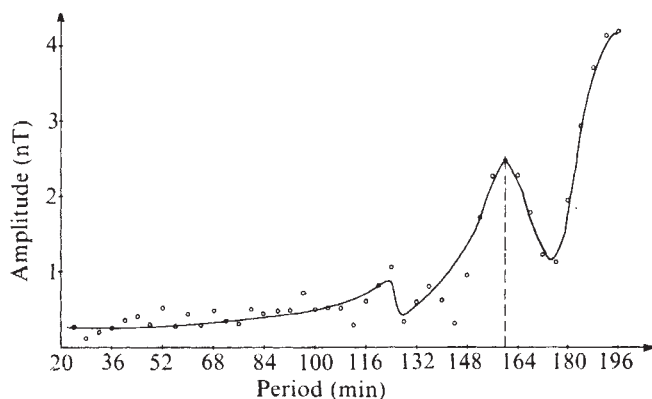
Such a connection means that magnetic field variations originating from the Sun's pulsation, which in the interplanetary space reaches as far as the magnetosphere, perturb the geomagnetic field. This can be observed on magnetic records in the observatories. Since the oscillation observed on the Sun's surface corresponds to magnetic variations of Gaussian magnitude, variations of several nT (10^{-5} G) amplitudes are possible on the Earth's surface.

During magnetically quiet cycles the magnetosphere is closed, there is no merging, and so no pulsation of solar origin can be observed. During magnetic storms, however, the noise level rises to such an extent that, although the merging exists, no significant amplitude could be detected around the above period.

We do not know whether spectrum lines investigated by the astrophysical observatories are insensitive to magnetic effects in a way that they are not affected by disturbances of the magnetosphere. If this were possible, then the possibility of a pulsation of a so far unknown terrestrial magnetospheric origin cannot be excluded. Hence the observed spectrum lines may be influenced by such a pulsation, which may have seemed to be of solar origin.

Thus, if the possibility that spectrum lines with 5,123.7 Å wavelength can be affected by geomagnetic field variations is excluded, then the magnetic variation with a period of 2 h 40 min

Fig. 2 Mean amplitude spectrum of the variations of the geomagnetic field for the cycle 9–11 November 1975.



originating from the magnetosphere presents an indirect proof of the Sun's oscillations. Thus the Sun with nearly homogeneous distribution of density has a radial mode fundamental oscillation within the range of the above period. At the same time this phenomenon may prove that the interplanetary and terrestrial magnetic field merge.

For a final conclusion to be reached, however, the observed spectrum line of solar origin must be examined for its sensitivity to variations of the geomagnetic field. If it is insensitive, the possibility of a magnetospheric effect can be excluded.

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Short-term influence of anthropogenic sources on tropospheric baseline lead

ESTIMATING tropospheric baseline lead requires relatively long collection periods using conventional high volume methods because the airborne concentrations are very low in comparison with levels found in urban areas. During such collections, anthropogenic sources of lead may be present for short periods leading to apparently high results, even at remote locations such as the mid-Pacific Ocean¹ and Antarctica². Significant geographical variations in soil-sized Pb particles were also related to anthropogenic sources affecting the distribution of lead in marine atmospheric particulates^{3,4}. We describe here investigations using furnace atomic absorption spectrometry (AAS) of the lead content in the atmosphere around Cape Grim, Tasmania.

High volume particulate collections give average results which do not account for atmospheric changes occurring over a few hours because of the collection times required. Limitations are also imposed by the sensitivity of the analytical technique for lead determination and the blank levels of filter media used. Furnace atomic absorption spectrometry (AAS) has been proposed as a suitable technique for the determination of background atmospheric lead particulates⁵, using the graphite furnace atomiser as the particulate collection device and taking advantage of the extremely high sensitivity for lead and small air sample volumes required. We have used graphite cups in the dual role of collection device and furnace atomiser, using micro air-sampling techniques based on earlier work^{6–8} to follow atmospheric lead concentration over short periods of time, 7 h (but 3–10 min in urban areas) using a consecutive series of individual samples. The graphite cups (RW1 grade Ringsdorf Werke) gave the unique advantage of a filter with a zero blank for lead which can be transported to and from the sampling location without contamination⁹.

The site for sampling was the Australian Baseline Atmospheric Pollution Station (Department of Science) at Cape Grim, North West Tasmania, located in the Southern Hemisphere as shown in Fig. 1a. The station is situated on the edge of a cliff, 90 m above the sea and receives western maritime winds which travel over the Southern Ocean at this latitude. But winds travelling around high pressure centres (counter-clockwise air flow in the Southern Hemisphere) and within 90° of north east may be subjected to anthropogenic lead sources from urban-industrial sources in Tasmania and the mainland of Australia (Fig. 1b).

A series of 15 consecutive samples were taken over five days (Table 1 and Fig. 2). Other measurements taken on site, over the

same period, were a high volume sample (Table 1), meteorological data (Fig. 2) and a comparison of hygroscopic (sea-salt) particles and non-hygroscopic particles per unit volume (Table 2).

Meteorological data for the period 14–16 May (Fig. 1) showed a steady easterly wind (low wind speed) proceeding towards Cape Grim from the urban-industrial centres along the northern coastal area of Tasmania. This apparently has a strong influence on the total particle concentration (Table 2). Between 16 and 17 May the wind reversed direction (easterly to westerly). Sampling of lead particulates was started then.

Samples 1–4 showed very high atmospheric lead concentrations which seemed to be anomalous at first examination. The reversal of the steady air mass, however, from the previous few days was associated with precipitation (rainfall) on 16 and 17 May and caused the atmospheric lead concentrations to be as high as typical urban levels. The influence of precipitation is also apparent from the particle data (Table 2).

Following the cessation of precipitation (sample 4) and the displacement of all impure air, the westerly winds brought very clean maritime air to Cape Grim and the atmospheric lead concentration fell to very low levels (samples 5–12). The total particle concentrations (Table 2) from late on 17 May until the middle of 21 May were the lowest for the whole period.

On 20 May a wind change to north–north westerly direction led to an increase in atmospheric lead concentration (samples 13–15) but in this instance directly following the wind change, implicating the influence of anthropogenic sources. This was also

Table 1 Atmospheric lead particulate concentrations at Cape Grim

Sample no.	Date May 1976	Sampling period (h)	Sampling time (min)	Pb ($\mu\text{g m}^{-3}$)
1	17	0758–1500	422	0.086
2	17	1558–2258	420	0.109
3	18	0000–0700	420	0.213
4	18	0835–1535	420	0.043
5	18	1603–2300	417	0.0044
6	19	0006–0710	424	0.0084
7	19	0906–1605	419	0.0014
8	19	1653–2355	422	0.0033
9	20	0017–0717	420	0.0032
10	20	0922–1928	606	0.0037
11	20–21	1945–0043	298	0.0011
12	21	0107–0818	421	0.0009
13	21	0907–1609	422	0.015
14	21–22	1708–0013	423	0.019
15	22	0016–0718	422	0.040
Mean	Samples 1–15 $0.037 \pm 0.059 \mu\text{g m}^{-3}$ Pb			
Mean	Samples 5–12 $0.0033 \pm 0.0024 \mu\text{g m}^{-3}$ Pb (base line)			

Analysis for Pb furnace AAS was performed using the Varian Techtron Model 63 CRA following addition of $2 \mu\text{l}$ $1,000 \mu\text{g ml}^{-1}$ H_3PO_4 to each cup and Pb standards in 20% HNO_3 (double distilled). The Model 63 CRA workhead was mounted in a Model 1200 AAS which was set to the 217.0 nm Pb resonance line and 1.0 nm slit. Non-atomic absorption was negligible. Detection limit for 10-l air sample was $0.0036 \mu\text{g m}^{-3}$ Pb and > 20 l air was sampled for baseline conditions, identified from on-site meteorological equipment. Total volume of air passed through each cup with a Millipore Type XX60 220 50 pump, was measured using an American Dry Type Test Meter (Model DTM 115) because flow-rates through individual cups were variable (range 0.028 – 0.31 l min^{-1}). During preparation each sample and blank cup was fired through the atomiser cycle for Pb to check that contamination was at blank level before placing in Teflon adapters and glass containers⁹ and transportation to sampling site. Dimensions of RW1 grade graphite cups are as described for other cups⁸.

High volume air sample (Kimoto HVA and Whatman GF/A glass-fibre filter) 0804 h 17 May–0717 h 22 May $0.0056 \pm 0.0011 \mu\text{g m}^{-3}$ Pb. Measurement of air flow-rate was made twice daily during the collection period and used to calculate the volume of air sampled ($9,692 \text{ m}^3$). Whatman GF/A filter was divided into two and extracted with 10 ml 20% HCl and 40 ml 40% HNO_3 (both high purity) in a Soxhlet extractor for 3 h. Solution was taken to near-dryness and made up with 10% HNO_3 to 50 ml after filtering. Average of 4 filters, treated similarly, was taken for blank. Pb was determined with Pye-Unicam Model SP1950 AAS incorporating automatic background correction and gave a detection limit of $0.0008 \mu\text{g m}^{-3}$ Pb (217.0 nm Pb resonance line), in this case.

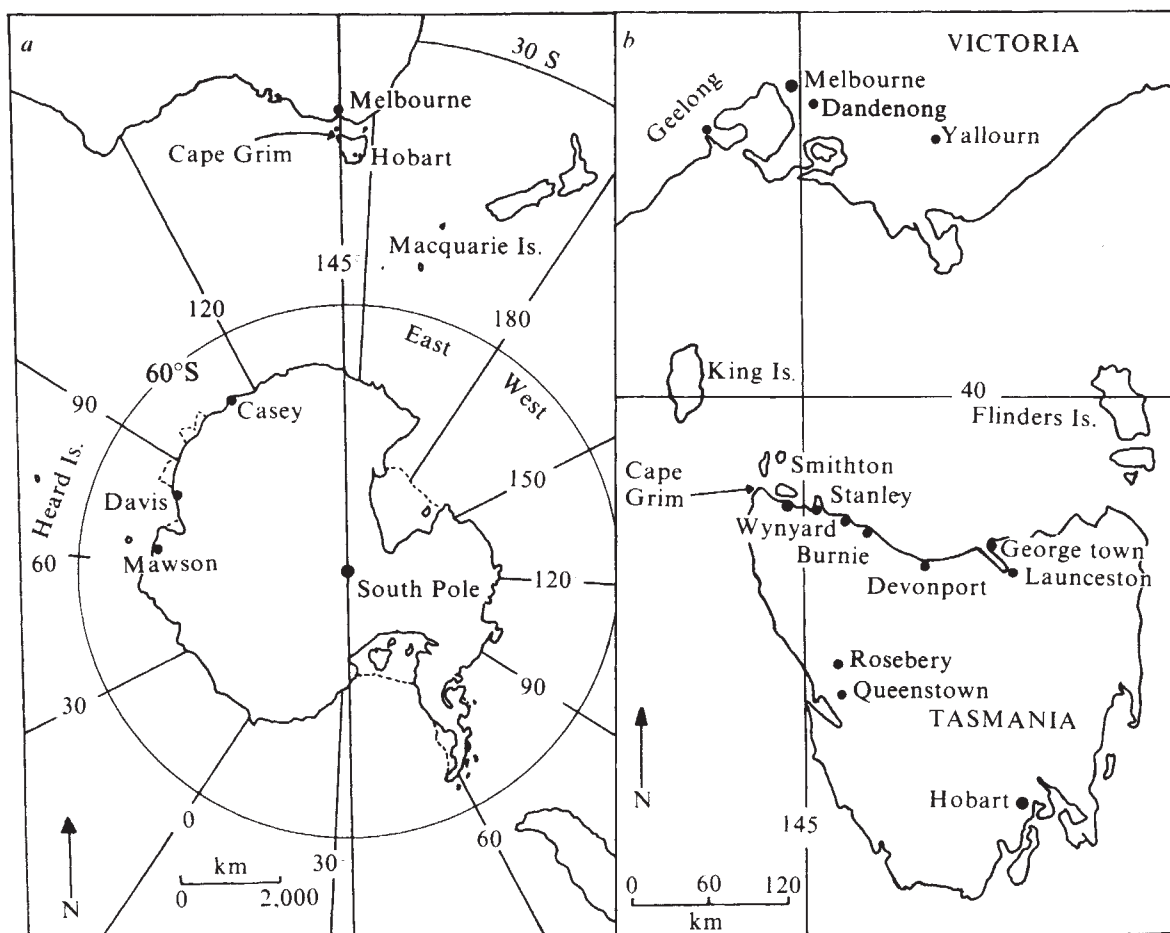


Fig. 1 Location of Cape Grim in relation to *a*, Southern Hemisphere and *b*, Urban and industrial centres of Tasmania and the mainland of Australia.

associated with a dramatic increase in total particle concentration (Table 2) compared with sea-salt particle concentration demonstrating the interaction of air from an urban origin with the background air and the importance of meteorological data in interpreting the atmospheric lead results.

The efficiency of graphite as a filtration medium may be judged by comparing the mean for samples 1–15 ($0.037 \mu\text{g m}^{-3}$ Pb) with the high volume sample result ($0.0056 \mu\text{g m}^{-3}$ Pb). Both figures include lead from anthropogenic and background origins and hence the high volume figure is not indicative of baseline atmospheric concentration. Glass-fibre filters as used in high volume samplers, are reported to be greater than 99% efficient for di-(2-ethylhexyl)phthalate particles ($0.3 \mu\text{m}$ in diameter) at a flow rate of 0.1 ml min^{-1} (ref. 10). Comparison of graphite with common filter media, including glass-fibre for urban atmospheric lead particulates, showed graphite to be a much more efficient filter material, presumably because it retains a larger proportion of sub- μm lead-containing particles¹¹. Studies of size distributions of airborne particles at various urban and non-urban locations are almost always multi-modal¹² and considerable proportions of particles can be in the size range 0.05 – $1.0 \mu\text{m}$. The size distribution of the smaller particles (Table 2) (including lead) has a mode between 0.01 – $0.05 \mu\text{m}$ diameter in polluted conditions and may be as great as $0.1 \mu\text{m}$ or larger in clean conditions (E. K. Bigg, personal communication). Sea-salt particles (Table 2), by comparison, have a modal diameter of about $1 \mu\text{m}$ and rarely have diameters below $0.3 \mu\text{m}$. This information supports the theory that the glass-fibre filter collection (Table 1) was not retaining a large proportion of sub- μm lead-containing particles. Particles of the order of $0.1 \mu\text{m}$ will have longer atmospheric residence times compared

with those less than $0.01 \mu\text{m}$ or greater than $10 \mu\text{m}$, which tend to agglomerate, be washed out, or become 'fall-out'.

The mean figure for samples 5–15 ($0.0033 \mu\text{g m}^{-3}$ and equivalent to 3.3 ng per SCM) describes the background atmospheric lead concentration at Cape Grim for the period sampled and compares in magnitude with data from the remote HASL Southern Hemisphere stations¹³ (Easter Island, Antarctica (Chilean stations), South Pole station) for the period 1966–1976.

Table 2 Nature of air particles at Cape Grim (comparison of hygroscopic with non-hygroscopic particles)

Date	8-h collection beginning at denoted hour			
	0000 (M/N)*	0600 (M/N)	1200 (M/N)	1800 (M/N)
May 1976				
14	0.6/448	0.67/–	0.67/480	0.69/560
15	0.66/–	0.60/576	–/–	–/–
16	2.9/432	3.1/–	3.2/–	3.6/272
17	3.4/344	–/–	–/–	4.7/144
18	4.4/–	4.2/–	4.8/160	3.8/–
19	3.6/154	–/–	–/–	3.6/164
20	2.4/150	–/–	–/256	1.8/–
21	2.6/–	3.6/128	3.7/–	4.1/936
22	–/–	–/–	–/–	3.4/126

Lead particulates are associated with non-hygroscopic particles (difference of sea-salt and total particles) but particles from urban sources are very often mixed. Sea-salt particles form about 0.1% (numerically) of particles in polluted conditions and strictly non-hygroscopic particles; 0.1–10%. Most non-sea-salt particles usually have a hygroscopic coating (E. K. Bigg, personal communication).

*M is salt particles cm^{-3} (hygroscopic) and N is total particles cm^{-3} (electrostatic precipitator) as weighted running mean over at least 24 h. (E. K. Bigg, personal communication.)

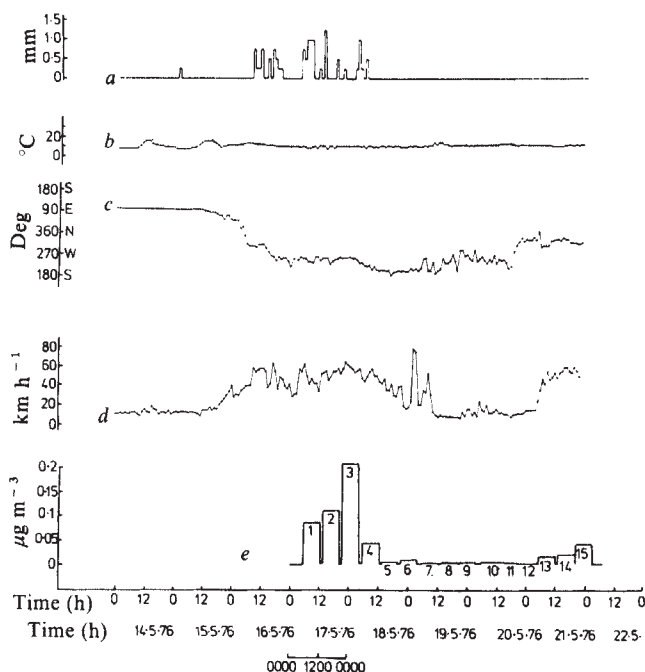


Fig. 2 Diurnal variations of atmospheric particulate lead, wind speed and direction, temperature and precipitation at Cape Grim. *a*, Precipitation for whole hour; *b*, temperature for minutes 6–9 of the hour; *c*, mean wind direction for minutes 3–6 of the hour; *d*, mean wind speed for minutes 0–3 of the hour; *e*, atmospheric particulate lead. (Data other than lead from E. K. Bigg.)

The latter (mean monthly composite high volume samples) are higher in many cases because they represent ambient air lead concentrations with probable anthropogenic contributions rather than background alone. Comparison with other data from carefully selected sampling periods at the South Pole station¹¹ (mean 0.63 ng per SCM, range <0.19–1.2 ng per SCM) shows the Cape Grim figure to be higher. A possible explanation for the higher Cape Grim atmospheric lead concentrations may be the higher collection efficiencies achieved thereby.

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Palaeoanisotropy in the upper mantle

CARTER¹ has reviewed advances in understanding the steady-state flow of rocks with particular reference to the behaviour and anisotropic alignment of rocks in the upper mantle. Some form of high-temperature creep is clearly responsible for the distribution of continents and their first-order structures². The difficulty is specifying the exact mechanism and the possible anisotropic alignments. Appropriate upper mantle temperatures and pressures can be achieved in the laboratory³, but any results must be extrapolated to rates of deformation lower by some five or six orders of magnitude. Carter *et al.*² conclude, although there are conflicting views⁴, that the syntectonic recrystallisation suggested by Avé Lallemant and Carter⁵ is the dominant deformational process. This will be most effective at the higher temperatures at the base of the lithosphere where crystals, orientated by the deformations, have their alignments fixed as the lithosphere cools. All the deformational processes suggested for the upper mantle are capable of orientating crystalline structures, and aligned azimuthal-anisotropy may be expected at any depth in the lithosphere.

It is difficult to make accurate observations of the upper mantle, but whenever the lithosphere has been examined in sufficient resolution it is found to be anisotropic. Occasionally upper mantle material can be examined in the laboratory. Peselnick *et al.*⁶ measure up to 7% velocity anisotropy in lherzolite xenoliths from the Sierra Nevada, which are estimated on compositional grounds to have originated at 50 km depth in the upper mantle. The only way *in situ* information can be obtained, however, is from seismic observations. Existing observations of azimuthal anisotropy in the upper mantle are of three types⁷ (1) P_n velocity anisotropy, widely observed beneath the oceanic crust, and now beneath the Rhinegraben⁸, could be the result of as little as 5 km of anisotropic material beneath the moho. (2) Velocity anisotropy of fundamental-mode surface-waves in the NAZCA Plate⁹ is attributed to anisotropy extending down possibly to the top of the low-velocity-zone at about 125 km depth. (3) Polarisation anomalies of second-mode surface-waves across Eurasia¹⁰ indicate at least 10 km of anisotropic material. These seismic observations establish anisotropy in the top few kilometres of the lithosphere, but, except for type (2) observed in one area only, give no indication of the vertical range.

There seem to be two main possibilities for the configuration of anisotropic alignments throughout the lithosphere in any given region. In uniform alignment, there will be little variation with depth of the alignment in the lithosphere, regardless of the tectonic history, if there is a deformation process which can modify the alignment, perhaps very slowly, to conform with the existing stress pattern over the past few million years.

In layered alignment, the alignment of the lithosphere will vary with depth if the alignment at the lower higher-temperature lithospheric boundary is permanently fixed as the lithosphere cools. This will result in the lithosphere having layers with different alignments corresponding to stress or flow patterns at some epoch in the past when the physical conditions were suitable for realignment to occur.

If syntectonic recrystallisation^{1,2,5} is the dominant alignment process and there is no subsequent deformation, the layered configuration will persist, and palaeoanisotropy in the upper mantle will be preserved over long periods of time. In regions of comparatively young lithosphere such as that beneath the oceans, where the forces acting on the lithosphere have been substantially the same throughout its history, the alignment will be uniform. Carter¹ finds that the plastic deformation processes of Hess¹¹ and Francis¹² give rise to similar fabrics as syntectonic recrystallisation, and remarks that the question of which process is dominant is now less important. But, the process which produces the existing alignments and its period of

activity, is still fundamental to the interpretation of the anisotropic configuration of the lithosphere.

We see that to achieve a substantial realignment by any of the various processes in the given physical conditions, the relative time-constant determines which configuration exists. It is difficult to believe that any *a priori* arguments derived from surface experiments will clearly distinguish between configurations resulting from slow deformations in the upper mantle acting over hundreds of millions of years. Observations of the presence, absence, and variation of anisotropic alignments at different depths beneath oceans and continents may, therefore, be crucial in deciding the deformation processes and the tectonic history of the lithospheric upper mantle.

Numerical calculations^{10,13,14} demonstrate that most seismological measurements are very little affected by even substantial amounts of anisotropy. The only diagnostic difference, between propagation through anisotropic and propagation through isotropic structures, is the coupling in the presence of anisotropy between polarisations in the radial-vertical plane and the transverse-horizontal direction. This coupling provides a technique for investigating deep anisotropy: body waves passing through anisotropic structures generate polarisation anomalies at each interface, which may be interpreted in terms of depth, thickness, orientation, and degree of anisotropy^{13,14}. These anomalies are likely to be subtle and difficult to observe but they provide probably the only means of examining the *in situ* palaeoanisotropy in the lower lithosphere.

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Giant haloes in mica

MUCH attention has been given to the origin of 'giant' pleochroic haloes found in a number of minerals, especially micas^{1–6}. Normal pleochroic haloes are due to the effects of radiation damage from α -particles emitted from small inclusions at the centre of each halo, and the largest radius of the halo is determined by the range of the α -particle of highest energy, which is that from ²¹²Po in the case of a thorium-rich inclusion. These have an initial energy of 8.78 MeV. The giant haloes seem to be of two kinds. In Gentry's sample⁷ ~40% have a discrete radius ~54 μ m and he reports them to be invariably associated with dense thorium haloes. This group appears properly to be associated with production by the well-known but rare (~1.3 $\times 10^{-4}$) pair of long range α -particle groups from ²¹²Po at ~10.5 MeV. The remaining giant haloes have a monotonic distribution in radius extending up to 110 μ m; they have been interpreted in terms of diverse processes such as extinct radiations of higher energy, proton knock-ons by α -particles,

knock-ons from neutrons, channelling, migration of the parent radioactive material, but we report one natural and simple explanation which seems not to have been considered.

The giant haloes (illustrated, for example, in ref. 4) are also outstanding in that there is a large inclusion. Such large inclusions in mica, which is well known for the readiness with which it may be cleaved, seem very likely to be surrounded by a cleavage crack lying in the natural crystallographic cleavage plane, and presumably filled principally with H₂O. The plane of the crack would pass close to the inclusion centre. Such a crack could well be initiated by changes in temperature or pressure at some stage during the life of the specimen, or possibly by swelling of the inclusion due to the high degree of radiation damage that it receives. If cracks radiating from the inclusion have propagated in planes approximately normal to the natural cleavage plane, as apparently happens in the haloes illustrated in ref. 3 and in Fig. 2 of ref. 4, then cracking parallel to the natural cleavage is extremely likely.

It seems reasonable that the surrounding crack in the cleavage plane could have an opening height, $h \geq 0.1 \mu$ m at the boundary of a large inclusion, and extend up to perhaps 50 μ m outwards in the case of the largest inclusions. (Cracks with smaller openings, could possibly heal gradually, if a little plastic deformation of the mica can occur, and they are not kept open by intrusion of solid particles. With larger openings, stabilisation of the opening by inflow of debris becomes more likely. The clearly visible cracks normal to the cleavage plane (shown in refs 3, 4) must have h well exceeding our suggested lower limit.)

Cracks such as we consider here would help extend the volume exposed to radiation damage in two ways: (1) by allowing α -particles entering or crossing the crack nearly parallel to the cleavage plane to travel a significant fraction of their range in H₂O; and (2) by enhancing the capabilities of diffusion of the radon isotopes in radioactive equilibrium with the inclusion, some of which will be liberated following the recoil at their formation from their parent radium nucleus.

To be effective in extending the range of the α -particles the crack has to possess sufficient height so that α -particle scattering in the water can be ignored. For this simplifying approximation to be adequate it is necessary that $h \geq 5 \times 10^{-4} x^{3/2}$ where x is the path length in H₂O and both h and x are measured in μ m. The range extension thus endowed to the α -particles is close to 50% of the distance travelled in H₂O since α -particle ranges in H₂O are close to twice those in both biotite and muscovite micas. The proportion of α -particle tracks stopping in the crack plane that have a range extension appropriate to maximum passage through H₂O will be of the order of $h/2a$, where a is the radius of the inclusion; this figure can, of course, be much larger than 1.3×10^{-4} which is the abundance of the naturally occurring 10.5 MeV long-range α -particles.

With a half-life of ~52s, ²²⁰Rn liberated from a thorium-rich inclusion has sufficient time to diffuse over the full extension of the volume of the crack, taking a diffusion coefficient equal to ~10⁻⁵ cm²s⁻¹, so that the polonium α -particles, in particular, should have an origin that is more extended than the volume of the inclusion. This is also true for the uranium-rich inclusions, where ²²²Rn has a half-life of 3.8 d. Such diffusion is very obvious in 'thorium stars' produced by radioactive impurities in nuclear emulsion. These are tracks of successive α -particles produced in the decay chain from single nuclei of ²²⁴Ra and ²²⁸Ra which have an abundance of ~3 $\times 10^4$ cm⁻³ in normal nuclear emulsion, Brown and Fowler, in ref. 8.

With each of these mechanisms the halo produced should not be spherical. It should have a radius of normal magnitude perpendicular to the cleavage plane. In the case of enhancement by α -particle propagation along the plane of the crack, the fully enhanced radius of the halo should be restricted to a band of thickness $\Delta Z \sim h$ the crack opening, whereas if enlarged by radon diffusion the enhancement would spread over a thickness ΔZ of the order of the range of the subsequent polonium α -particles. Quantitative information about the form of the

halo in this third dimension would be most valuable in attempting to determine which mechanism is in fact important in the generation of these giant haoles.

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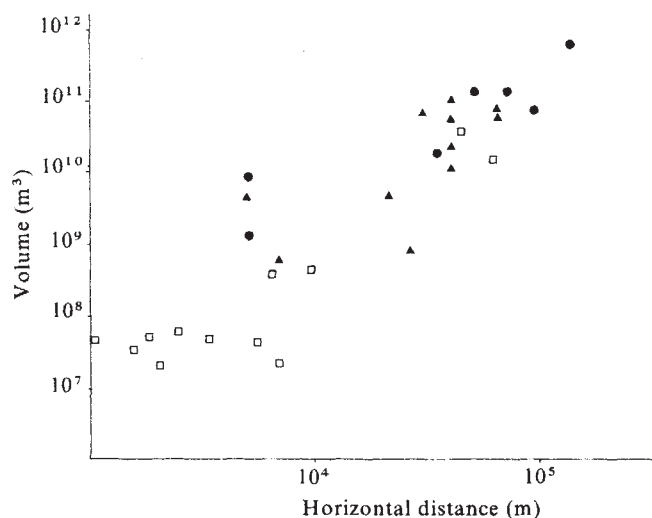
Mobility of pyroclastic flows

THE mobility of pyroclastic flows has been described as spectacular¹, in particular because of their ability to surmount obstacles in their path. It is relatively easy to estimate the minimum velocities required to climb a given height, if frictional losses are ignored. We have used data on well documented large cold rock avalanches to estimate the magnitude of frictional losses, and used these estimates to calculate velocities for some large pyroclastic flows. We argue that frictional losses in large pyroclastic flows must be comparable with, or less than, those in the most mobile rock avalanches, and calculate from data on heights climbed that velocities of large pyroclastic flows may be very high, up to 140 m s^{-1} . We also conclude that the height of the eruption column giving rise to a large pyroclastic flow need only be a few hundred metres in some cases.

It is difficult to investigate the mobilities of large pyroclastic flows, such as those forming ignimbrites, since none has ever been observed in eruption. Only a few relatively small *nuée ardente* eruptions have been observed directly. Some of the largest ignimbrites have volumes of the order of 100 km^3 , extend for horizontal distances over 100 km (ref. 2), have climbed obstacles up to 600 m in height and have been estimated to travel at speeds up to 100 m s^{-1} (ref. 3).

The scanty previous literature on mobility of pyroclastic flows has been summarised by Sparks³ and Miller and Smith⁴.

Fig. 1 Horizontal distance travelled for well documented large rock avalanches and ignimbrites plotted against volume. □, rock avalanches; ▲, Central Andes ignimbrites; ●, other ignimbrites.



We have obtained some further data from the Central Andes, where three ignimbrites are known which have climbed obstacles up to 350 m high². The way in which pyroclastic flows surmount obstacles has been disputed³. Recent observations on the upward concentrations of pumice clasts in ignimbrites imply high flow densities³; that is, pyroclastic flows may behave in a similar way to large rock avalanches.

For rock avalanches greater than about 10^{-3} km^3 , Hsü showed that mobility is related to the volume—the largest being the most mobile⁴. Hsü explained the unusual mobility of large rock avalanches in terms of fluidisation of fine debris by entrapped air; Shreve in contrast suggested that rock avalanches travel on a cushion of trapped, compressed air⁵. Whatever the mechanism, the efficiency of the process seems to increase with the size of the moving body.

Many ignimbrites have volumes comparable with or greater than the largest known rock avalanches, some having volumes in excess of 100 km^3 (ref. 6). The very high mobility of the pyroclastic flows giving rise to such deposits can be inferred from Fig. 1, where horizontal distance travelled is plotted against volume for pyroclastic flows and large rock avalanches. The large volume ignimbrites plot in a distinctly more mobile field than all but the largest of the rock avalanches.

Although there are a few direct measurements on the velocities of small *nuée ardentes*—up to 60 m s^{-1} (refs 7 and 8)—the velocities of the much larger volume pyroclastic flows can only be inferred from their climbing ability. Simple calculations, based on the conversion from potential to kinetic energy, can be done which relate the horizontal velocity to the height climbed. By this means, Sparks showed that a minimum velocity of the order of 100 m s^{-1} is required for a pyroclastic flow to climb a 600 m high obstacle³. Such calculations take no account of frictional losses. We have obtained crude estimates of these losses in pyroclastic flows by looking at data for much better known rock avalanches. In the case of a body of rock travelling down one slope and up an opposing one, an estimate of the overall percentage frictional loss (F) can be derived, from the interconversion of potential energy and kinetic energy, by including frictional loss in the appropriate formulae

$$\begin{array}{lcl} \text{kinetic energy gained} & \text{descent} & \text{potential energy lost} \\ \frac{1}{2}mv^2 & = & \left(\frac{100-F}{100}\right)mgh_1 \end{array}$$

$$\begin{array}{lcl} \text{potential energy gained} & \text{ascent} & \text{kinetic energy lost} \\ mgh_2 & = & \left(\frac{100-F}{100}\right)\frac{1}{2}mv^2 \end{array}$$

where m = mass; v = velocity, F = percentage frictional loss, h_1 = height lost; h_2 = height gained

$$\text{from which} \quad F = 100 \left(1 - \left(\frac{h_2}{h_1}\right)^{\frac{1}{2}}\right)\%$$

Clearly, the calculated value of F will be increased if the flow travels a large horizontal distance between the two slopes, or if the main direction of travel is oblique to the slope climbed. Relevant data for the best known rock avalanches are listed in Table 1. In the more mobile of these F is between 40 and 60%. In Fig. 2 we have plotted the theoretical relationship between horizontal velocity and height climbed for values of F between 0 and 60% (solid lines).

Taking the data summarised in Fig. 1 into account, large pyroclastic flows are likely to have values of F comparable to, or even lower than, the most mobile rock avalanches, that is, in the range 30–40%. On this basis, the velocities required for the obstacle climbing ignimbrites plotted on Fig. 2 would be in the range 85 to 140 m s^{-1} . These values are realistic: the errors not

Table 1 Height data and values of F for documented large rock avalanches

Name	Volume (m ³)	Height descended h_1 (m)	Height climbed h_2 (m)	F (%)	Remarks
Elm ⁴	1.1×10^7	450–560	100	50–55	
Little Tahoma Peak ⁹	1.1×10^7	1,250–1,890	40–90	75–85	Climbed oblique slope
Sherman ¹⁰	2.8×10^7	600	140	50	
Madison ¹¹	2.9×10^7	400	120	45	
Frank ⁵	3.7×10^7	760–920	120	60–65	
Silver Reef ⁵	2.2×10^8	1,000	45	80	Travelled a long horizontal distance
Blackhawk ⁵	2.8×10^8	1,220	60	80	Travelled a long horizontal distance
Saidmarreh ¹¹	4.3×10^9	1,520	460–610	35–45	

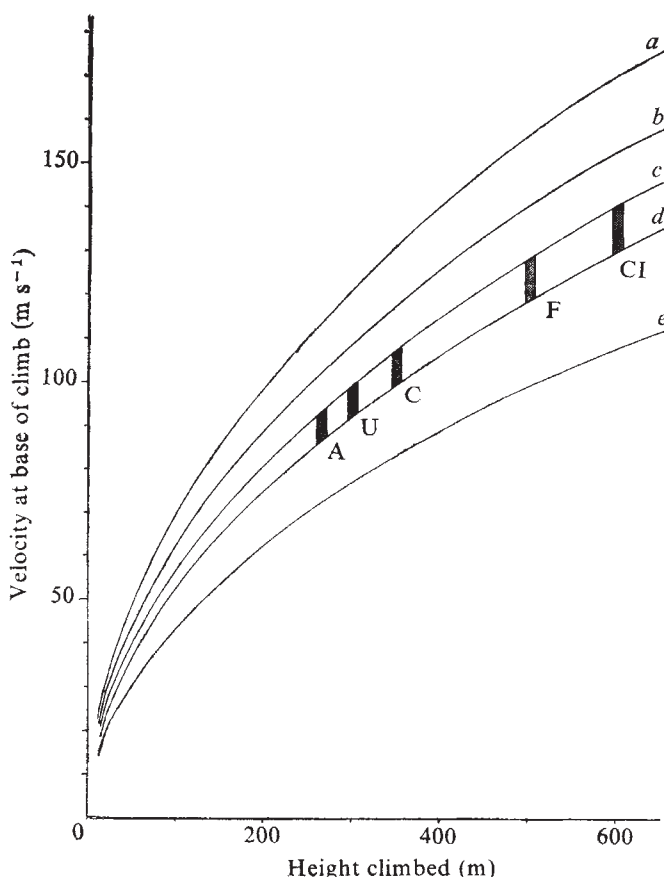
taken into account in our simple model would only increase the values of F , and therefore the velocities required. Such high velocities have two important implications.

First, the emplacement of even a very large ignimbrite sheet could take place in a very short time, that is minutes rather than hours. This is particularly relevant in considering the mechanisms of transport of pyroclastic flows, and the origin of textures within them, such as the sorting of pumice fragments. Second, if values for F for pyroclastic flows giving rise to ignimbrites were around 30–40%, indicating efficient lubrication of the flow by fluidised fine grained material, it is not necessary to invoke collapse from eruption columns of exceptional height

Fig. 2 Theoretical relationship between horizontal velocity at base of climb and height climbed for values of F : a, $F = 60\%$; b, $F = 50\%$; c, $F = 40\%$; d, $F = 30\%$; e, $F = 0\%$; using the

expression $V = 10 \left(\frac{2gh_2}{100F} \right)^{\frac{1}{2}}$. Also plotted are estimated velocity

ranges for individual ignimbrites assuming values of F between 30 and 40%: A, Aniakchak¹; U, Ujina²; C, Carcote²; F, Fisher¹; CI, Campanian³ and Ito³.



to account for the high speeds. For example, collapse from a comparatively modest column 400–500 m high would be sufficient to account for the mobility of the Ujina ignimbrite. In other cases, where the pyroclastic flow travelled a substantial distance horizontally before surmounting an obstacle, the frictional losses would have been greater, and therefore the mobility of the flow must have been achieved by collapse from an eruption column of comparatively great height.

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Ratio of prey to predators in community food webs

WHETHER the diversity of resources limits the diversity of consumers, and specifically, whether the number of kinds of prey limits the number of kinds of predators, has been of continuing interest in theoretical ecology and wildlife management^{1–3}. Food webs from the ecological literature were collected in machine readable form to study this question empirically. We report here that in community food webs, the ratio of the number of kinds of prey to the number of kinds of predators seems to be constant, near 3/4. This invariance has not been noticed in earlier studies of individual cases.

Before analysis, food webs were characterised as one of three types—community, sink and source. Community food webs describe all kinds of organisms (possibly restricted to some location, size or taxa) in a habitat, without reference to the eating relations among them. Sink food webs describe all the prey taken by a set of one or more selected predators, plus all the prey taken by the prey of those predators, and so on. Source food webs describe all the predators on a set of one or more selected prey organisms, plus all the predators on those predators, and so on. Sink and source food webs, hypothetical or schematic constructions, and avowedly incomplete, partial or tentative food webs were excluded from further study. Fourteen community food webs were thus selected. The complete data and individual cases will be discussed elsewhere⁴. When the report of a food web contained ambiguous or uncertain information about a

feeding relation, the web was included in two versions, one based only on the unambiguous information and the other incorporating the additional uncertain or probable eating relations. The analysis presented here based on all versions, makes no claim that the data points are statistically independent and attaches no probability values to the statistics calculated.

The food webs describe the diets or predators not of individual organisms but of kinds of organisms. A 'kind of organism' may be a stage in the life cycle or a size class within a single species, or a collection of functionally or taxonomically related species, according to the practice of the original report. The numbers in the following analyses refer to these ecologically defined kinds of organisms, not necessarily to any conventional taxonomic unit. A predator is defined as a kind of organism that consumes at least one kind of organism included in the food web. A prey is defined as a kind of organism that is consumed by at least one kind of organism in the food web. Some kinds of organisms may be both predators and prey.

In community food webs, the number m of prey is very nearly proportional to the number n of predators (Fig. 1). A least squares regression of m against n gives

$$m = 1.79 + 0.71 n \quad (1)$$

The sample standard deviation of the regression coefficient is 0.07 and the linear correlation coefficient between m and n is 0.90. The standard error of estimate, or sample standard deviation from regression, is 4.62. As is obvious from Fig. 1,

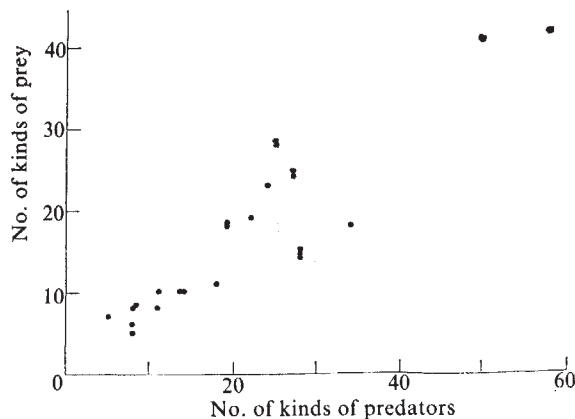


Fig. 1 The number of kinds of prey and the number of kinds of predators in community food web versions.

the regression may be well approximated by a straight line through the origin. The least squares regression is

$$m = 0.77 n \quad (2)$$

The proportionality between the number of prey and the number of predators in Fig. 1 is based on 24 versions of 14 food webs reported over a period of decades. When the food webs were collected and encoded it was not known that such a simplicity would emerge. It therefore seems likely that this invariance in the proportions of predators and prey represents a fact about nature, rather than an artefact of collusion or convention.

Given that the proportion of prey to predators is a scale-invariant feature of community food webs, the proportion can be predicted quantitatively from other facts. For a given food web with m prey and n predators, let A be the number of predator-prey couples. (If X eats Y and Y eats X , the couples (X,Y) and (Y,X) are counted as distinct. If X eats X , (X,X) also counts as a couple. In the conventional graphical representation of a food web, A is the number of directed arrows from prey to predator.) Then within any food web

$$\begin{aligned} A &= (\text{average prey per predator}) \times n \\ &= (\text{average predators per prey}) \times m \end{aligned} \quad (3)$$

The grand mean over all 24 community food web versions, weighting each food web equally, of the average prey per predator is 2.418; the grand mean of the average predators per prey is 3.199. If these means apply to each food web, then substitution into equation (3) predicts

$$m/n = 2.418/3.199 = 0.756 \quad (4)$$

which differs trivially from the least squares regression in equation (2).

The simplicity of the argument from the proportionality between m and n to equation (4) may raise a suspicion that its success depends on an arithmetical fact rather than on the observed invariance of proportions of predators and prey in nature. A numerical example disproves this suspicion. Suppose a sample of community food webs consisted of two food webs. Suppose the first food web matrix had $m_1=8$ prey, $n_1=6$ predators, and $A_1=19.2$ predator-prey couples (neglecting the requirement that A_1 be integer for the sake of argument). Then its (average predators per prey)₁ is 2.4 and its (average prey per predator)₁ is 3.2. Suppose the second food web matrix had $m_2=4$, $n_2=10$, and $A_2=16$. Then its (average predators per prey)₂ is 4.0 and (average prey per predator)₂ is 1.6. Then the grand mean over both food webs of the average predators per prey is 3.2 and the grand mean of the average prey per predator is 2.4, which are close enough to the observed. But the straight line through the pairs (n, m) satisfies $m=14-n$. Only because nature assures a constant proportion of prey to predators do the grand mean of the average predators per prey and the grand mean of the average prey per predator apply to all food webs.

If the ratio of prey to predators in community food webs is a constant of the order of 3/4, then dividing equation (3) by n leads to the prediction that a regression (Fig. 2) of average prey per predator against average predators per prey should be a straight line through the origin with slope 3/4. The regression coefficient of a straight line through the origin is 0.69, not far from 3/4.

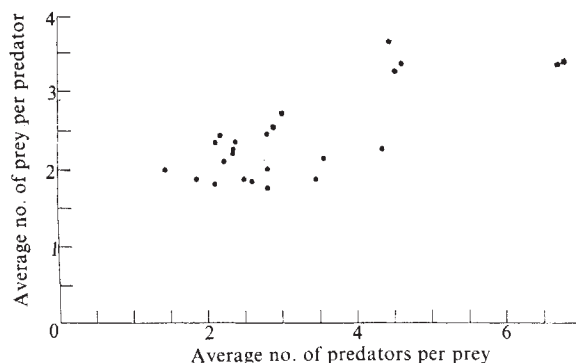


Fig. 2 The average number of kinds of prey per kind of predator and the average number of kinds of predators per kind of prey in community food web versions.

In conclusion, in community food webs, the number of kinds of prey, as operationally defined by field ecologists, approximates 3/4 the number of kinds of predators. This results from the study only of an ensemble of food webs, rather than of individual cases.

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Inhibition of drinking by intrahypothalamic administration of morphine

MORPHINE inhibits the release of acetylcholine (ACh) within both the peripheral^{1–4} and central^{5–9} nervous systems. This would suggest that a major effect of acute morphine treatment should be inhibition of behaviour mediated by central cholinergic transmission, but few behavioural demonstrations of this antagonism exist. We report here that intrahypothalamic injections of equimolar concentrations of morphine effectively antagonise the muscarinic-cholinergic drinking response^{10–13} elicited by injection of 4.0 nmol of carbachol into the hypothalamus. In addition, intracranial (i.c.) injections of morphine also blocked drinking elicited by several other dipsogenic stimuli, while not affecting eating. The results suggest that morphine not only inhibits the release of ACh but also may block the postsynaptic muscarinic receptor. This inhibitory effect of i.c. morphine may, furthermore, provide a simple behavioural screening test for central opiate activity.

The effects of intrahypothalamic applications of morphine on water consumption were investigated in two experiments involving 44 adult male Sprague-Dawley rats. In each experiment, stainless steel cannulae (24 gauge) were stereotactically implanted into the perifornical hypothalamus¹⁴ (PFH) of anaesthetised (Nembutal, 40 mg per kg body weight intraperitoneally) rats. Each subject was allowed at least one week to recover from surgery before testing was initiated. Drugs were administered i.c. as free bases in 1- μ l volumes through a 31-gauge hypodermic needle directly connected to a 50- μ l syringe. A repeating dispenser (Hamilton, PB600–1) was used to ensure reliability of injection volumes. Before any tests of morphine, stable baseline drinking to the dipsogenic stimuli was ensured across several days of testing. Ground rat chow and water intake were measured after 1-h periods of both control and experimental treatment conditions. Tests of drug antagonism were conducted by administering an equimolar dose of the antagonist to the same brain area 5 min before the injection of the agonist.

In the first experiment, 28 rats were randomly divided into three groups. Eating and drinking were assessed in each group after the control injection of saline and the subsequent application of carbachol (Fig. 1). As previously reported¹³, carbachol elicited significantly more eating ($F(1,25) = 15.35$, $P < 0.01$) and drinking ($F(1,25) = 67.72$, $P < 0.01$) than the control manipulation.

On the following day, the antagonistic effect of morphine on carbachol-elicited drinking was investigated. The reversibility of morphine antagonism of drinking was also tested in another group by the i.c. injection of the narcotic antagonist, naloxone, 5 min before the morphine treatment. Although there were no significant differences in food intake between the three groups (Table 1), there was a significant overall effect on water consumption ($F(2,25) = 13.17$, $P < 0.01$). Post hoc *t* tests indicated no difference in drinking between the saline pretreated carbachol (SSC) and naloxone-morphine pretreated carbachol (NMC) groups, while each of these groups differed from the saline-morphine pretreated carbachol (SMC) group ($P < 0.01$). Thus, i.c. morphine blocked carbachol-elicited drinking, while not significantly affecting eating. Furthermore, this antagonism of drinking was reversed by pretreatment with naloxone.

Since cholinergic influences have also been implicated in the mediation of deprivation-induced drinking¹⁵, 18 of the above rats were deprived of food and water for 24 h and tested for

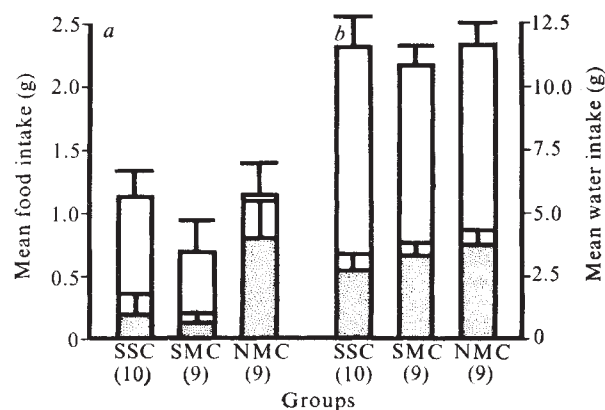


Fig. 1 Mean food (a) and water (b) intake ($\pm$ s.e.m.) by rats during the 1-h following the injection of carbachol (4.0 nmol) and the preceding control injection of 1 μ l of normal saline (stippled area). This test was conducted after the rats had received three previous daily injections of carbachol and saline and represents the animals' response during the fourth trial. Group designations refer to the morphine test, which was conducted on the following day: S, saline; C, carbachol; N, naloxone; M, morphine. Numbers in parentheses are number of animals tested.

morphine antagonism of drinking and eating. That i.c. morphine effectively antagonised drinking while not significantly affecting eating (Table 2) indicates that this adipsic effect is not due to nonspecific depression of behaviour.

In the second experiment, 16 experimentally-naive rats were randomly assigned to two groups to assess the effect of i.c. morphine across several other paradigms of drinking. Since morphine has been reported to inhibit the uptake of carbachol into brain slices¹⁵, carbachol was administered before morphine in the first test. Although carbachol was injected well before morphine and undoubtedly had been taken up by central neurones (as shown by a normal drinking latency of 5 min), drinking was still significantly reduced by the opiate (Table 3). The second test examined the effects of i.c. morphine treatment on drinking elicited by hypertonic saline¹³. Again i.c. morphine effectively obviated the drinking response to this dipsogenic challenge (Table 3). Pretreatment with equimolar doses of morphine also antagonised drinking elicited by the potent dipsogenic peptide, angiotensin II¹⁶ (Table 3).

To provide additional control for nonspecific effects of morphine on consummatory behaviour, two additional tests were conducted. Since morphine seemed to depress eating somewhat in previous experiments (Tables 1 and 2), the effects of

Table 1 Effects of intrahypothalamic morphine and naloxone pretreatments on carbachol-elicited eating and drinking

Group	N	Food intake (g per h)	P	Water intake (g per h)	P
SSC	10	1.1 $\pm$ 0.3	NS	12.0 $\pm$ 1.1	0.01
SMC	9	0.5 $\pm$ 0.3		2.7 $\pm$ 1.5	
NMC	9	0.6 $\pm$ 0.2		9.1 $\pm$ 1.7	

Mean food and water intake in grams ($\pm$ s.e.m.) by rats during 1 h following the injection of carbachol (4.0 nmol). Group SSC received injections (1 μ l) of normal saline 10 and 5 min before carbachol, while groups SMC and NMC were injected with saline (1 μ l) followed by morphine (4.0 nmol) or naloxone (4.0 nmol) followed by morphine (4.0 nmol), respectively, 10 and 5 min before carbachol. Although there were no overall differences in food intake, group SMC drank significantly less water than group SSC or group NMC. There was no significant difference in water intake between groups SSC and NMC. Statistical inferences were made using analysis variance techniques with post hoc comparisons made by *t* tests. NS, not significant.

Table 2 Effects of intrahypothalamic morphine treatment on eating and drinking elicited by 24 h food and water deprivation

Treatment	N	Food intake (g per h)	P	Water intake (g per h)	P
Saline	9	5.0±0.7	NS	6.2±0.8	0.01
Morphine	9	3.4±0.8		1.2±0.4	

Following the test of morphine inhibition of carbachol-elicited eating and drinking (Table 1), 18 rats were deprived of food and water for 24 h. The saline group received 1 µl of normal saline, while the morphine group was injected with 4.0 nmol of morphine 5 min before being presented with food and water. Intake in grams (±s.e.m.), was assessed during the 1-h period following the initial presentation of food and water. Although there was no difference between groups in food intake, morphine-treated animals drank significantly (*t* test) less water than did those given saline. NS, not significant.

i.c. morphine on eating and drinking elicited by 48-h food deprivation were investigated. It is well known that eating is reduced when drinking is severely inhibited. Therefore, the purpose of this test was to maintain hydration at near normal values with drinking being elicited by increased thirst subsequent to food consumption. As indicated in Table 3, there was no difference in food intake between the saline and morphine groups. Water intake, however, was still severely inhibited by the morphine treatment. To assess the effects of pretreatment with a non-addicting, non-analgesic stereoisomer, dextrophan was substituted for morphine in this final test. To control for the possibility of tolerance to the opiates, the groups were reversed with the dextrophan group never having been injected with opiates. In contrast to the previously-observed effects of morphine, i.c. dextrophan did not reduce either eating or drinking in these satiated rats (Table 3).

These experiments demonstrate a potent inhibitory effect of

Table 3 Effects of intrahypothalamic opiates on eating and drinking elicited by various dipsogenic stimuli

Group	N	Food intake (g per h)	P	Water intake (g per h)	P
C-S	8	1.4±0.5	NS	10.1±0.2	0.01
C-M	8	1.1±0.7		2.2±0.8	
S-HS	8	0.1±0.1	NS	11.4±1.5	0.01
M-HS	8	0.4±0.3		2.4±1.2	
S-A	8	0.5±0.3	NS	19.8±2.2	0.01
M-A	8	0.6±0.3		7.2±1.8	
FD-S	8	6.1±0.8	NS	5.8±1.3	0.01
FD-M	8	7.4±0.8		0.9±0.2	
S-C	8	1.1±0.4	NS	9.5±2.3	NS
D-C	8	1.6±0.3		11.0±1.9	

Mean food and water intake was measured in grams (±s.e.m.) during 1-h periods following various dipsogenic stimuli. During the first test, intracranial (i.c.) injections of carbachol (C; 4.0 nmol) were administered 10 min before the i.c. injection (1 µl) of saline (S) or 4.0 nmol morphine (M) with the rats being presented with food and water 5 min after the last injection. Six days later, i.c. M (4.0 nmol) or S (1 µl) was administered 5 min before the injection (2 ml; subcutaneously) by hypertonic (10%) saline (HS). After a 3-d latency, the antagonistic effects of i.c. M (4.0 nmol) or S (1 µl) were assessed when administered 5 min before i.c. angiotensin II (A; 4.0 nmol). Six days later, the rats were food-deprived (FD) for 48 h and presented with food and water 5 min after the injection (i.c.) of M (4.0 nmol) or S (1 µl). The last test, conducted 6 d later, assessed the effects of i.c. dextrophan (D; 4.0 nmol) and S (1 µl) as pretreatments (5 min) on eating and drinking elicited by 4.0 nmol (i.c.) C. Statistical significance is based on *t* tests. NS, not significant.

centrally-applied morphine across several paradigms of drinking. This effect was not due to nonspecific behavioural disruption, since the rats were alert and ate amounts of food comparable to the control groups. Furthermore, the antagonism of drinking seemed to be due to the narcotic properties of morphine, since it was reversed by pretreatment with naloxone. This hypothesis is strengthened by the lack of effect observed when the stereoisomer, dextrophan, was substituted for morphine.

These inhibitory effects of morphine on drinking are similar to results previously reported following administration of atropine or scopolamine and may reflect antimuscarinic cholinergic activity. Although pretreatments with atropine have been reported to block drinking elicited by carbachol¹¹, hypertonic saline¹³, water deprivation¹³, and schedule-induced polydipsia¹⁷, atropine is apparently not nearly as potent as morphine in paradigms that do not involve exogenous cholinergic dipsogens. Furthermore, atropine has been reported to be ineffective against drinking elicited by angiotensin II¹⁸, while pretreatment with morphine effectively blocked this behaviour. A possible difference between the central nervous system activity of morphine and atropine may be the effect of these drugs on the release of ACh. Thus morphine, which has been extensively reported to inhibit the release of ACh, may exert its antagonism of drinking through both the inhibition of release and postsynaptic muscarinic blockade. Thus, drinking in paradigms not involving exogenous cholinergic dipsogens, such as water deprivation, are not as readily antagonised by atropine. Conversely, morphine will readily inhibit the release of ACh and thus block the cholinergic components of deprivation-induced drinking. The reduction of drinking following i.c. angiotensin by morphine pretreatment may furthermore reflect a cholinergic component in the mediation of this behaviour¹⁸.

Antimuscarinic cholinergic effects of morphine have been suggested by several observations. The muscarinic cholinergic agonist, pilocarpine, stimulates transmission in the superior cervical ganglion of the cat¹⁹, and this effect, furthermore, is blocked by atropine as well as by morphine¹⁹. The observation that muscarinic receptor blockers reduce symptoms of precipitated morphine withdrawal²⁰ also suggests antimuscarinic actions of the drug. An additional indication of antimuscarinic activity of morphine is provided by the observation that tolerance to epileptiform electroencephalogram patterns following microinjections of morphine into the amygdala is accompanied by cross tolerance to scopolamine²¹.

We are extending these investigations to other opiate and opioid drugs and investigating the effect of systemically-administered morphine on these various dipsogenic challenges. It is possible that these tests may lead to a simple behavioural test of central narcotic activity.

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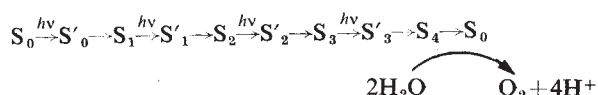
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EPR signals in chloroplasts responding to illumination sequence of four flashes

THE use of water as the electron donor and the consequent evolution of oxygen is one of the most important properties of photosynthesis by higher plants and algae. The properties of the enzyme systems involved in water oxidation are essentially unknown, however. Two new electron paramagnetic resonance (EPR) signals observed at low temperature in oxygen evolving chloroplasts are described here. The signals disappear and appear in response to short flash illumination, before freezing, of the chloroplasts, presumably due to changes in oxidation state of the components giving rise to the signals. The changes occur in response to a cycle of four flashes; it is suggested that they may reflect the oxidation states of the oxygen evolving system of the chloroplasts.

Manganese may be an important component of the oxygen evolving system¹, but attempts to observe changes in the redox state of manganese associated with oxygen evolution have not been successful. Kinetic measurements^{2,3} have shown that when chloroplasts are illuminated with short intense flashes of light, each of which causes only a single electron to be transported by each photosynthetic reaction centre, oxygen is evolved with a periodicity of four flashes. These experiments suggest that each water oxidising enzyme system accumulates four oxidising equivalents, one with each turnover of the photosystem II reaction centre, before reacting with water. This observation may be formalised in the 'S' state hypothesis of Kok *et al.*³



In kinetic experiments oxygen evolution normally shows a peak after the first three flashes, and then with a periodicity of four flashes, indicating that the distribution of the 'S' states is not random in the dark. S_1 and S_0 are thought to be most stable in the dark, with S_1 predominating in most experiments⁴. The light induced progression between the 'S' states represents a change in redox state of the proposed enzyme, each step involving a single electron oxidation. As this enzyme may be expected to contain a transition metal ion complex it seems likely that these changes could be detected by EPR spectroscopy. Several studies of chloroplast electron transport using EPR and flash illumination have been reported but none has identified signals corresponding to the 'S' states⁵. Both optical and nuclear magnetic resonance (NMR) spectrometry have been used in attempts to identify the 'S' state components. Pulles *et al.*⁶ and Mathis and Haveman⁷ have reported optical changes at 310 nm responding to flash illumination with a periodicity of four. The chemical nature of the component involved is unknown. Wydrzynski *et al.*^{8,9} observed changes in the relaxation rate of water protons showing response to flash illumination with a periodicity of four using NMR spectrometry: they presented evidence that their results are consistent with the participation of manganese in the flash-induced changes in the relaxation rate of water protons.

We have used flash illumination at room temperature followed by rapid freezing to 90 K in an attempt to trap the 'S' states, and low temperature (4.2–30 K) EPR spectrometry to detect new EPR signals showing a response to flash number, which would be expected from the oxygen evolving enzyme system, or a chloroplast component reflecting the redox state of this system.

Figure 1 shows the EPR spectra of a chloroplast preparation in the dark and after exposure to 0, 1, 2, 3, or 4, 20- μ s flashes from a 25-J xenon-arc lamp, the multiple flashes were spaced at 200-ms intervals. The samples were frozen within 1 s using an isopentane bath at 90 K and stored at 77 K until the spectra were recorded. The spectra show a signal in the dark at $g = 3.09$.

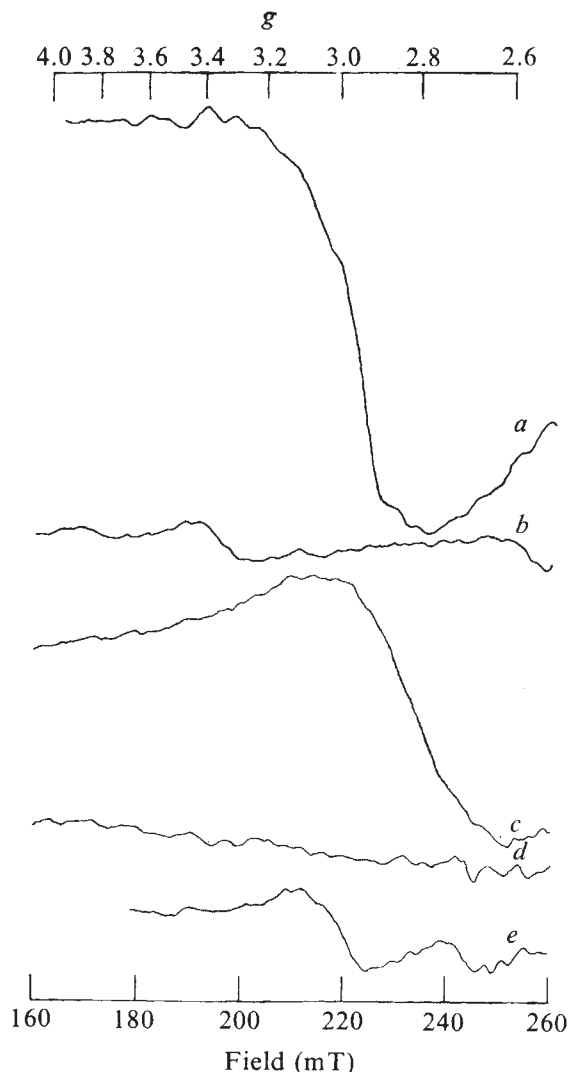


Fig. 1 EPR spectra of broken spinach chloroplasts in the $g = 3.0$ region. Broken chloroplasts were prepared in a Mn-free medium containing 1mM EDTA^{15,16}. The chloroplasts which exhibited an uncoupled rate of oxygen evolution of 306 μ mol per mg chlorophyll per h were kept in the dark on ice for 0.5 h. The chlorophyll concentration was 1.55 mg ml⁻¹. EPR spectra were recorded as described previously^{17,18}. The conditions for each spectrum were as follows. Frequency 9.189 GHz, power 20 mW, modulation amplitude 1mT, scan rate 100 mT min⁻¹, gain 500, temperature 10 K. The samples were illuminated with a, 0; b, 1; c, 2; d, 3 and e, 4 flashes. The spectra were recorded in the dark.

After one flash there is essentially no signal in the $g = 3.0$ region. After a second flash a large signal appears centred at $g = 2.79$. The properties of this signal suggest that it arises from a different redox state to that giving rise to the dark signal. After the third flash there is again no signal while after the fourth flash, which in the 'S' scheme should regenerate the dark state, the original signal reappears at $g = 3.07$. This signal is, however, usually rather smaller than the initial dark signal.

These signals might be considered to correspond to the S_1 , S_2 , S_3 and S_0 states of Kok's model. S_4 would not be seen as it reacts rapidly with water¹. The rate of this reaction might, however, control the extent of regeneration of S_1 by the fourth flash. Some decay of each stage will also occur between flashes, this and other problems well documented for the kinetic experiments will rapidly result in a loss of periodicity and the ability to detect the signals.

As described above, the response of these signals to flash illumination strongly suggests that they reflect the oxidation state of the water oxidising enzyme system. The EPR properties

of these signals are extremely unusual, however, making it experimentally very difficult to investigate the role of the components giving rise to them, or to attribute them to a specific chloroplast component. In all preparations in which we have observed these signals they show strong orientation effects, similar to those observed in EPR studies of single crystals¹⁰. Fig. 2 shows the effect of rotating a dark sample (*a* in Fig. 1) in the EPR cavity. If the sample is oriented to give maximal signal size (0°) and then rotated the signal disappears (40°) and then reappears (180°). At intermediate positions the signal size, linewidth and g value differ. Figure 2*c* (50°) shows this effect. We have found that in order to be sure that a sample does or does not have a signal the spectrum must be recorded every 10° through 180° , 18 spectra per sample. The spectra in Fig. 1 show the maximum signal size for 0, 1, 2, 3 and 4 flashes from a set of 18 spectra for each sample. The apparent g value also changes with orientation as is observed in a single crystal. The g value and linewidth of the dark signal is also temperature dependent. Figure 3 shows the temperature dependence of the dark signal. The signal has a higher g value at lower temperatures with $g = 3.38$ at 3.8 K, $g = 3.27$ at 11.7 K and $g = 3.14$ at 19.8 K. The linewidth also changes with temperature becoming narrower at lower temperatures being 70 G at 3.8 K, 120 G at 11.7 K and 170 G at 19.8 K with the sample orientation used in Fig. 3. The temperature dependence of the g value of the dark signal suggests that the signal arises from a ferromagnetic complex. Ferromagnetism is most commonly observed with iron; however, several other transition metals, but not apparently

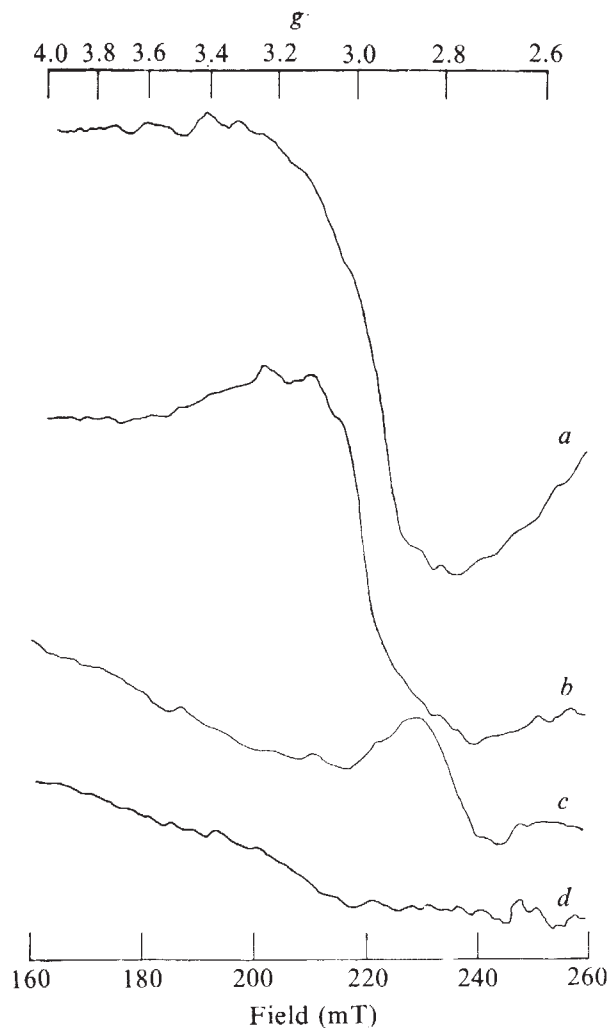


Fig. 2 Effect of sample position on the dark EPR signal in broken spinach chloroplasts. Sample and conditions as for Fig. 1*a*. The relative orientations of the samples were *a*, 0° ; *b*, 180° ; *c*, 50° and *d*, 40° .

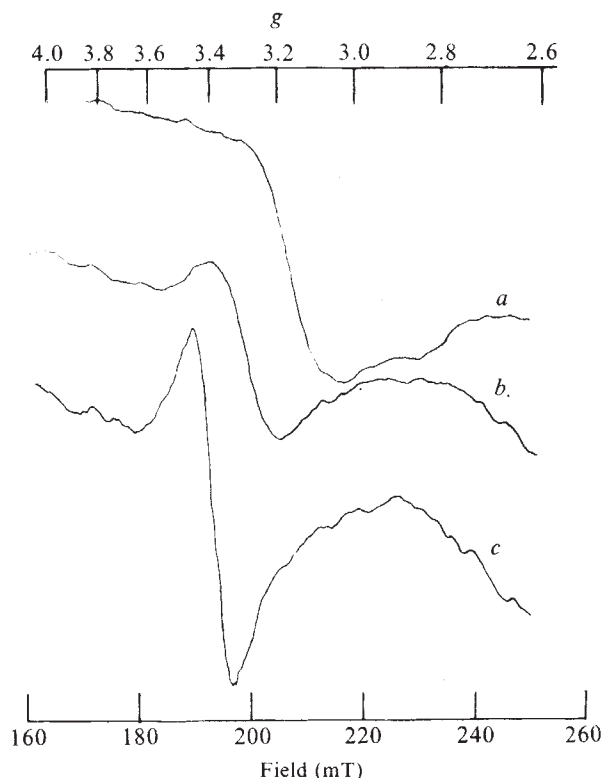


Fig. 3 Effect of temperature on the dark EPR signal in broken spinach chloroplasts. Conditions and samples as in Fig. 1*a* except that the temperatures were *a*, 19.8 K; *b*, 11.7 K; *c*, 3.8 K.

manganese, can show this property¹¹. Ferromagnetism is a cooperative effect seen only when two or more atoms are in close proximity. Oriented ferromagnetic EPR signals have been reported from nerve tissue and nucleic acid preparations^{12,13}. These signals were observed at room temperature, however, unlike the signals reported here which could not be observed above 40 K. We have not observed any temperature dependence of the g value of the signal seen after two flashes, indicating that the redox state giving rise to this signal is not ferromagnetic and is therefore different from the state giving rise to the dark signal.

Although the properties of these signals are unusual, the results presented here have been obtained consistently mainly in spinach chloroplasts but also in lettuce and pea chloroplasts. They are not seen in chloroplasts which have been washed with Tris, (0.8 M Tris-HCl pH 8.0 for 10 min at 2° , the thylakoids were then centrifuged and resuspended in the original medium), a procedure which inactivates the water oxidation system¹⁴, or in photosystem I particles prepared with the anionic detergent Triton X-100. The association of these signals with chloroplasts and their absence from photosystem I particles, together with the observation that they are lost when the oxygen evolving system is destroyed, suggest that the signals are associated with photosystem II. EPR signals responding to single turnover flash illumination might be associated with either the electron donor or acceptor sides of photosystem II. But signals associated with the acceptor would be expected to show a binary, two flash, response cycle, as the acceptor is thought to be a quinone which would be fully reduced in two steps. Signals associated with the water-oxidising system would be expected to show a four-flash response cycle. The signals reported here apparently show a four flash cycle as two states with different signals and two states without signals are observed. These changes would correspond to a model of the oxygen-evolving complex in which the electrons are removed sequentially from a single complex. They would not fit models in which electrons are removed from isolated centres each of which undergoes the same redox reaction.

We conclude that these signals probably reflect the oxidation state of the water oxidising enzyme system; future experiments

should lead to the identification of the component giving rise to these signals and the properties of this component which lead to orientation of the signal.

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Effects of foetal haemoglobin on susceptibility of red cells to *Plasmodium falciparum*

HIGH gene frequencies for the sickling disorders and β thalassaemia may be due to the relative protection against *Plasmodium falciparum* malaria which has been afforded to heterozygous carriers. In sickling disorders the properties of the abnormal haemoglobin may be responsible for this phenomenon²⁻⁴ but the red cells of adult β thalassaemia heterozygotes contain reduced amounts of normal haemoglobin⁵ and are indistinguishable from normal red cells with respect to the rates of invasion and growth of *P. falciparum* (G.P., D.J.W. and R.J.M., in preparation). Hence it is not clear how the β thalassaemia polymorphism has been maintained. There is a possible explanation, however. At birth red cells contain mainly foetal haemoglobin (Hb F); adult haemoglobin (Hb A) replaces Hb F during the first year of life. The rate of decline of Hb F production during this period is retarded in infants heterozygous for β thalassaemia as compared with normal infants⁶. If the presence of Hb F were to protect red cells against *P. falciparum*, β thalassaemia carriers aged 6-18 months might be at an advantage when passive immunity to *P. falciparum* is waning but active immunity is not fully established and mortality from malaria is high⁷. To test this hypothesis we have compared the rates of invasion and growth of *P. falciparum* in red cells containing Hb A with those containing Hb F using a modification of the *in vitro* culture system⁸⁻¹⁰.

Samples of 5 ml of blood containing synchronous late ring stages of *P. falciparum* were collected into heparin (10 IU ml⁻¹) and layered onto a 6 x 2 cm column of Whatman CF11 cellulose in phosphate-buffered saline (PBS). The

leukocyte-depleted red cells were washed through the column into 2-l Ehrlenmeyer flasks with 100 ml supplemented medium 199⁸. The flasks were gassed with 5% CO₂-95% air and incubated at 37 °C with gentle shaking. After about 24 h, when the parasites had grown to the schizont stage, the red cells were recovered by centrifugation and reconstituted to the original 5 ml volume with medium 199. Plasmagel 2.5 ml (3% w/v gelatin; Bellon) was added and the cells were allowed to sediment for 15 min at 37 °C to concentrate the schizont-infected cells in the upper layer. The latter were then suspended in a mixture of 3 parts supplemented medium 199 and 1 part non-immune AB serum. The uninfected red cells which were to be examined for the rate of parasite invasion and growth were collected into heparin, washed three times in PBS, the buffy coat removed and added to an appropriate volume of the parasitised red cell suspension so that the final concentration was approximately 80 x 10³ cells per μ l with 2-5% schizont-infected cells. The mixture was plated into the wells of a microtissue culture plate (250 μ l per well) and incubated in 5% CO₂-air at 37 °C without shaking. At various times blood films were made and either stained with Giemsa or treated by the acid-elution method¹¹⁻¹³ modified to demonstrate Hb F-containing cells while at the same time allowing adequate morphological characterisation of parasites.

P. falciparum preferentially invades young, metabolically active red cells¹⁴. As this study was designed to compare the rates of invasion and growth *in vitro* in cells containing Hb A with those containing Hb F, and as these cells might be of different metabolic ages, a preliminary series of experiments was carried out to examine the effect of cell age on the rates of invasion and growth of *P. falciparum* in the *in vitro* system. Normal red cells were fractionated by age^{15,16} and each fraction was mixed separately with infected cells. In each case the rate of invasion of the younger red cell population was significantly greater than that of the

Table 1 Invasion and development of *P. falciparum* in normal red cells of different ages

Cell fraction	Invasion		Developmental stages of 100 parasites per cell fraction							
	Reticulocytes (%)	Parasites (%)	R		S		T		L	
			S	L	S	L	S	L	G	A
Whole blood	0.4	5.0	0	3	40	30	11	1	2	13
Top	2.5	7.9	0	2	39	32	11	2	1	13
Middle	0.6	3.6	0	4	36	29	14	3	0	14
Bottom	0.2	3.3	0	3	36	24	16	1	2	18
Whole blood	2.4	5.1	0	1	31	37	14	1	1	15
Top	9.6	8.9	0	3	40	26	10	2	0	19
Middle	1.5	4.5	0	2	42	24	10	2	0	20
Bottom	0.1	3.2	0	0	49	21	10	1	0	19

Uninfected adult red cells were centrifuged at 2,500g for 1 h at 4 °C. Fractions of approximately 0.2 ml were removed from the top, middle and bottom of the column of red cells and from whole blood. Reticulocyte counts were done to confirm separation on the basis of age. Suspensions of schizont-infected cells were added to each fraction and the mixtures were incubated as described in the text. The percentage of ring forms in each fraction arising from a fixed inoculum of schizonts is shown for two experiments. At least 1,000 red cells were examined from each fraction. In each case a χ^2 test showed a significantly greater percentage of parasites in the top (young) cell as compared with the bottom fraction ($P < 0.01$). To assess parasite development smears were made after 53 h in culture and the maturity of parasites in 100 singly infected cells in each fraction was assessed. Rings (R) were forms without pigment, trophozoites (T) had a single clump of pigment and a single nucleus, schizonts (S) had more than one nucleus, gametocytes (G) had diffuse pigment, and abnormal forms (A) were those in which there was no nuclear-cytoplasmic differentiation on staining and which were pyknotic. The rings, trophozoites and schizonts were further subdivided into small (S) and large (L) forms. There was no significant difference in the pattern of development of the parasites between the various cell fractions.

older cells (Table 1). The further growth and development of the parasites in the different aged cell populations were assessed both by morphological examination (Table 1) and ^3H -isoleucine incorporation^{17,18}. Parasite growth assessed by either method was similar in each cell fraction. Thus in this *in vitro* system the parasites preferentially invade young metabolically active cells but there is virtually no difference in the rates of development in red cells of differing ages.

To determine whether Hb F has any effect on invasion of red cells by *P. falciparum*, the relative rates of invasion of Hb A and Hb F-containing cells were compared. Hb A-containing cells were obtained from normal adults. Hb F-containing cells were derived from four sources—normal newborn infants, infants aged 3–6 months, homozygotes for the British type of hereditary persistence of foetal haemoglobin (HPFH)¹⁹ and heterozygotes for the African form of HPFH²⁰. The choice of these Hb F-containing cells was based on the following rationale. The red cells of newborn infants contain mainly Hb F and are metabolically younger than those of normal adults²¹; by making an artificial mixture of newborn-infant and adult cells it would be possible to compare the rates of invasion of two red cell populations of different metabolic ages with Hb F in the 'younger' cells. In the blood of infants aged 3–6 months Hb F production has almost ceased and the newly-produced red cells contain almost entirely Hb A; in the same blood sample it would be possible to examine the relative rates of invasion of Hb A and Hb F-containing cells, in this case the latter being the metabolically older population. The red cells of homozygotes for the British form of HPFH contain increased amounts of Hb F which is unevenly distributed, some cells carrying mainly Hb F and others mainly Hb A¹⁸. Since the metabolic ages of these populations are similar it would be possible to study the relative invasion rates of Hb F and Hb A-containing cell populations of the same metabolic age in the same blood sample. The red cells in the African form of HPFH, apart from their increased levels of Hb F, are similar in

every way to those of normal adults¹⁹. The results are summarised in Table 2. In the mixture of newborn and adult red cells the Hb F-containing cells were preferentially invaded, in the red cells of the 3–6-month old infants there were significantly more parasites in the Hb A-containing cells, and in the red cells of the British HPFH homozygotes the Hb F and Hb A-containing populations were equally invaded. The rates of invasion of the African HPFH cells were identical to those of normal adult cells in parallel cultures. These experiments show that Hb F has no direct effect on the rate of invasion of red cells by *P. falciparum* and provide further evidence that invasion is a cell-age related process.

Table 3 Development of *P. falciparum* in cells containing varying amounts of foetal haemoglobin

Blood type	Cell type	Developmental stages of 100 parasites per group							
		R		T		S		G	A
		S	L	S	L	S	L		
Cord	A	0	0	25	58	8	5	2	2
	F	0	3	64	23	3	0	2	5
	A	0	1	29	48	17	2	0	3
	F	0	3	78	14	0	0	0	5
$\beta^0/\delta\beta$ Thalassaemia	A	8	10	44	28	6	0	0	4
	F	34	29	25	9	0	0	0	3
	A	10	30	25	27	7	0	0	1
	F	42	38	17	1	0	0	0	2
British HPFH	A	3	21	31	34	8	0	1	2
	I	14	26	28	20	6	0	0	6
	F	26	10	27	18	1	0	0	18
	A	21	5	4	8	30	19	2	1
African HPFH	I	45	14	4	11	20	3	0	3
	F	46	5	8	17	13	7	0	4
	A	1	6	27	20	19	9	0	18
	F	1	10	33	20	9	3	0	24
Infant	A	0	3	45	34	7	1	0	10
	F	0	4	54	24	1	0	1	16
	A	21	43	14	9	11	3	0	0
	I	22	68	8	0	2	0	0	0
	F	24	67	7	2	0	0	0	0
	A	10	31	33	12	9	0	0	5
	I	30	30	28	8	0	0	0	4
	F	28	34	28	4	0	0	0	6

Experimental methods are described in the text and the designation of cell type and the development stages of the parasites are the same as those in Table 1. All cells were cultured in the same wells except for the African HPFH and Hb A-containing control cells which were in parallel. There is a significant retardation of development of parasites in the Hb F-containing cells in each case.

A further series of experiments was carried out to compare the development of *P. falciparum* in Hb F-containing cells with that in cells which contain mainly Hb A. In addition to the cells used in the experiments described above a mixture of normal adult cells and cells from a compound $\beta^0/\delta\beta$ thalassaemia heterozygote which contain almost 100% Hb F²² were studied. The cells were infected *in vitro* and at various times smears were made from the cultures and a comparison of the maturity of parasites in the Hb A and Hb F-containing cells was made. The results of representative experiments are summarised in Table 3. There was a significant reduction in the number of mature forms of *P. falciparum* in the Hb F-containing cells regardless of their source or age. Hence the presence of Hb F in red cells causes retardation of growth of *P. falciparum* *in vitro*. Since it was observed in red cells containing Hb F obtained from both infants and adults with several different genetic conditions, this phenomenon is probably related to

Table 2 Invasion by *P. falciparum* of red cells containing varying amounts of foetal haemoglobin

Cell type	Source of Hb F-containing cells						F/A
	Adult	Cord	British HPFH	African HPFH	Infant		
	Parasites (%)	F/A	Parasites (%)	F/A	Parasites (%)	F/A	
A	5.2		10.8		7.4		
I	—	2.3	8.9	0.9	2.9	1.0	0.3
F	12.1		10.1		2.4		
A	5.1		9.6		4.3		
I	—	2.6	9.0	1.1	1.6	0.9	0.2
F	13.2		10.2		0.8		
A	5.5		4.3		12.2		
I	—	1.8	4.9	1.1	7.0	1.0	0.5
F	9.9		4.9		5.8		

Experimental conditions as described in the text. Where Hb A and Hb F-containing cells were present in the same culture, smears were stained by a modification of the acid elution technique to distinguish between the following groups of cells:— those containing predominantly Hb A where the haemoglobin was completely eluted (A), cells containing Hbs A and F (intermediate or I cells) and cells containing mainly Hb F (F cells)¹³. Since the mixture of adult and cord cells was artificial the group of I cells has been omitted. The African HPFH and adult control cells were grown in parallel and not in the same wells, as the acid elution technique does not readily distinguish African HPFH cells from those containing Hb A in an artificial mixture. In these cases the intermediate (I) group is omitted. At least 2,000 cells were counted in each experiment. The difference in parasitisation of the A and F cells in the adult/cord and infant cell experiments was significant at the 1% level. There was no significant difference between the parasite counts in the A and F populations in the British or African HPFH red cells.

the presence of Hb F and not to other metabolic properties of the red cells. Further evidence that this is the case was obtained in those experiments in which it was possible to define red cells which contained intermediate amounts of Hb F (Table 3). The degree of retardation of growth of *P. falciparum* in these cells was approximately intermediate between that in cells which contained predominantly Hb A or Hb F suggesting that the degree of retardation of growth of the parasite is broadly correlated with the level of Hb F in the red cell. Since the phenomenon was observed in the red cells of the African HPFH heterozygote, it seems likely that growth retardation can result from as little as 7 pg Hb F per cell.

Whether Hb F causes relative retardation of growth and development of *P. falciparum* by its high oxygen affinity, different primary structure from that of Hb A, or by another mechanism, remains to be determined. Whatever the mechanism this phenomenon, taken together with the retardation of the rate of decline of Hb F production over the first year of life in infants heterozygous for β thalassaemia, offers a possible mechanism for the maintenance of the β thalassaemia polymorphism. Any property of the red cell which causes even minor growth retardation or interferes with synchronisation of development of the parasite might provide some degree of protection, particularly at a stage of development when passive immunity is waning and active immunity is not established. Further data on the levels of Hb F which are required for protection, and on the intracellular levels of Hb F in such heterozygous infants during their first year is required to test this hypothesis. Its particular attraction is that because the rate of decline of Hb F is retarded in infants with β chain structural haemoglobin variants as well as those with β thalassaemia⁶, it provides a unifying mechanism for the high gene frequencies of the β -chain haemoglobinopathies. It does not, of course, exclude the operation of other protective mechanisms such as have been proposed for cells containing Hb S¹⁻⁴.

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Further link between complement activation and blood coagulation

EVIDENCE for interactions between the complement and haemostatic systems has come from two lines of research—blood platelets have been shown to interact with various complement components¹⁻⁶, and more ambiguous results have been obtained with respect to the role of complement in endotoxin shock and the Schwartzman reaction⁷⁻¹³. We report here that the activated complement component C3b triggers a marked increase of tissue thromboplastin (factor III) activity in cultured human monocytes. Differential counting and non-specific esterase staining¹⁴ of the final preparations regularly revealed more than 85% monocytes.

After isolation and incubation as described in Table 1, the cells were rinsed 10 times with sterile saline and mechanically displaced with a rubber policeman into 1 ml of Veronal-buffered saline¹⁵. Tissue thromboplastin activity was tested as before¹⁶. Procoagulant activity required the presence of coagulation factor VII and was inactivated rapidly by phospholipase C¹⁷ and by a monospecific antiserum against tissue thromboplastin^{18,19}. The procoagulant was thus identified as tissue thromboplastin. Guinea pig complement factor C3b was generated and purified as described by Nicholson *et al.*²⁰.

Table 1 summarises data from five experiments. C3b gives a marked increase in tissue thromboplastin activity of isolated monocytes cultured in the presence or absence of serum. This increase was of the same order of magnitude as that seen after endotoxin addition (data not shown). Cycloheximide (Sigma) (final concentration 10-20 $\mu\text{g ml}^{-1}$) inhibited the increase completely; thus protein synthesis was probably required. The effect of C3b was significant even at the lowest concentration

Table 1 Effect of C3b on tissue thromboplastin activity of human monocytes

Experiment	Additions ($\mu\text{g ml}^{-1}$)	Serum (20%)	Activity (units per mg protein)	Increase* (%)
1	C3b (0.5)	+	41.7	256
	C3b (0.5) + cycloheximide (20)	+	11.7	0
	None (control)	+	11.7	
2	C3b (12.5)	+	37.0	348
	C3b (12.5)	—	18.7	127
	None (control)	+	8.2	
3	C3b (15)	+	20.6	131
	C3b (30)	+	25.7	189
	None (control)	+	8.9	
4	C3b (30)	—	15.5	78
	None (control)	—	8.7	
5	C3b (30) + endotoxin (50)	+	69.3	1,055
	C3b (30)	+	56.6	843
	None (control)	+	6.0	

Human monocytes were isolated from mixed buffy coats by a modification of the Bøyum method²¹ including dextran sedimentation, two Ficoll-Paque (Pharmacia), gradients and several differential centrifugations. The cells adhering to the Petri dishes (Nunc), were selected by washing away non-adherent cells. Incubations lasted for 5 h. A standard preparation of human brain thromboplastin that clotted normal citrated plasma in 13 s was taken to contain 100 units ml^{-1} and dilutions of this preparation were used to establish a standard curve. Protein was determined by the Lowry method²². The endotoxin was *Escherichia coli* 055:135 type B (Difco). Each value is the mean of determinations in triplicate on duplicate cultures. The test samples had protein concentrations of 117-726 $\mu\text{g ml}^{-1}$ and the actual clotting times recorded were 18.7-38.8 s in C3b-stimulated samples and 41.3-68.8 s in controls. The clotting times were converted to thromboplastin units by means of the standard curve and normalised to units per mg protein.

*Control values subtracted.

tested ($0.5 \mu\text{g ml}^{-1}$). The apparent lack of a dose-response relationship is caused by the varying magnitude of the response of the different cell preparations used in the various experiments. In experiment 3 (Table 1) where different aliquots of the same cell preparation were exposed to two different levels of C3b, the higher dose gave the higher response. Unstimulated monocytes cultured for 5 h had very low activities (Table 1). C3b alone did not influence the assay system (tested up to $30 \mu\text{g ml}^{-1}$).

Isolated monocytes develop increased procoagulant activity when stimulated by endotoxin²³. This phenomenon probably requires both mRNA and protein synthesis (H.P. and A.C.A., in preparation) and is seen in monocyte cultures virtually free from blood platelets. Granulocytes and non-adherent mononuclear cells are essentially unresponsive (data not shown).

Human monocytes and other macrophages have receptors for C3b^{24,25}, and C3b is known to induce release of lysosomal enzymes from macrophages²⁶. The production of C3 by by normal macrophages²⁷ makes possible a positive feed back cycle²⁸. This link between complement activation and the extrinsic blood coagulation system has pathogenetic implications in the initiation of disseminated intravascular coagulation and possibly of thrombosis, which are under study.

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Induction of autophagy by amino-acid deprivation in perfused rat liver

AUTOPHAGOCYTOSIS is generally regarded as an important mechanism for ridding the cell of injured or unwanted cytoplasmic constituents and for degrading normal components in response to energy needs^{1,2}. Autophagy has been noted in HeLa cells deprived of foetal calf serum and amino acids³. It

has also been seen in rat liver after glucagon administration^{4, 5}, during nutritional deprivation^{7, 8} and in diabetes^{9, 10}. Thus autophagy could have a physiological role in the maintenance of body amino acid pools, although there is no direct experimental evidence for this. We reported previously that rates of proteolysis, and lysosomal sizes are seen to increase when rat livers are cyclically perfused with an unsupplemented medium and decrease in response to added amino acids¹¹⁻¹³. We strongly suspected that autophagy was the underlying event, but the nature of the process has been difficult to define¹³. The possibility was considered that its expression was self-limited by the accumulation of amino acids from endogenous proteo-

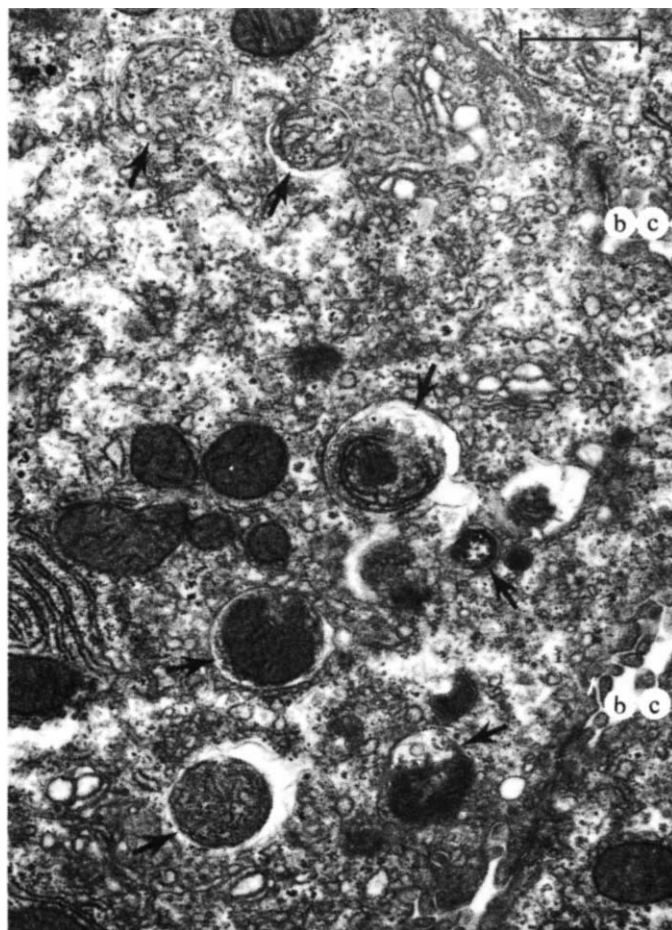


Fig. 1 Electron micrograph of a portion of a hepatocyte, adjacent to two bile capillaries (bc), from a liver perfused in the single-pass mode for 40 min with medium not supplemented with amino acids. After perfusion, livers were flushed with 10 ml of cooled perfusate plasma followed by 40 ml (in 4 min) of Karnovsky's glutaraldehyde-paraformaldehyde fixative², diluted 1:7 with 0.1 M sodium cacodylate (pH 7.2); 1-mm cubes then were cut from the major lobes and fixation continued with full-strength Karnovsky's medium for 3-4 h in an ice bath; the tissues were post-fixed in 1.33% osmium tetroxide, buffered with s-collidine (pH 7.4) for 2 h at ice temperature. The blocks were imbedded in epoxy resin (Durcupan ACM, Fluka) and thin sections cut with a Sorvall Porter-Blum MT 2-B ultramicrotome from an area containing predominantly mid-zonal cells²⁵. The sections were double-stained with uranyl acetate and either lead citrate or lead hydroxide before examination with an RCA-EMU-IV electron microscope. Numerous autophagic vacuoles are evident (arrows). Smooth endoplasmic reticulum, glycogen, a mitochondrion, and a portion of rough endoplasmic reticulum are especially well preserved within the vacuoles. Several of the autophagic vacuoles are limited by a double membrane throughout a significant portion of their circumference. In general, the hepatocytes seem to be in good condition. Mitochondrial swelling is not evident nor are there any large cytoplasmic vacuoles. There is some vesiculation which probably represents enlarged elements of the Golgi complex and which may play a part in the autophagic process.

lysis. We now show that when this accumulation is prevented by perfusing livers in the single-pass mode, autophagic changes appear that are morphologically indistinguishable from those occurring after maximal doses of glucagon⁴⁻⁸.

Livers from fed male rats of the Lewis strain (Microbiological Associates), weighing 120–150 g, were perfused *in situ* with a medium consisting of freshly washed bovine or sheep red cells suspended in Krebs–Ringer bicarbonate buffer (0.27 v/v) containing 3% bovine plasma albumin (Pentex) and 10 mM glucose. Amino acid levels in the perfusate plasma were reduced to 2.5% of normal rat plasma values as determined by direct analysis¹⁴. Perfusions were carried out as described previously¹⁵ except that in all single-pass runs the outflow from the livers was not returned to the perfusion flasks¹⁶.

Perfusion of livers in the single-pass mode with a medium depleted of amino acids evoked a striking increase in autophagy (Figs 1 and 2) that attained maximal intensity by 20 min and was clearly evident as early as 5 min (data not shown). Although autophagic vacuoles were widely scattered throughout the cell, they were more numerous in the vicinity of Golgi vesicles. The variety of identifiable cytoplasmic inclusions was fairly typical of the contents of glucagon-induced vacuoles¹⁷. The one exception was glycogen; while it was a frequent lysosomal constituent in our experiments, it has not been noted consistently in glucagon studies^{4-6,17}. This difference undoubtedly reflects the glycogen content of the cell at the time autophagy was underway. In most of these reports, the animals were starved and residual glycogen was undoubtedly lost through the glycogenolytic action of glucagon (through cyclic AMP). Although amino acid deficiency *per se* does not, in our experience, enhance glycogenolysis, we, nevertheless, considered the possibility that the albumin used in the medium might have carried substances capable of stimulating adenyl cyclase and autophagy. Analysis of tissue samples showed that cyclic AMP was not elevated, however (Table 1).

Effects of amino acid deficiency on the fractional cytoplasmic volumes of typical autophagic vacuoles are given in Fig. 2. With maximal deprivation, the fractional volume of autophagic vacuoles, 1.6%, was considerably larger than peak values after glucagon¹⁶, but compared closely with estimates after diabetes¹⁰ and in neonatal rat livers at the time of maximal autophagy¹⁸. In other respects, however, our findings are similar to those seen with glucagon. The cross-sectional dimensions of lysosomal profiles, which were up to 1.8 μm (Fig. 3), are in basic agreement with the data of Deter *et al.*⁵—we also noted, as they did, a significant decrease in the fractional volume of typical dense bodies (shaded fraction, Fig. 2). The addition of a 10 $\times$ physiological mixture of amino acids during single-pass perfusions completely prevented this autophagic increase and even reduced values below those in unperfused liver (Fig. 2). When the amino acids were added after autophagy was fully manifest (20 min), the vacuoles regressed almost completely with a half-time of 8–9 min (data not shown). This agrees closely with the time required to reverse the enhancement of lysosomal osmotic sensitivity in the perfused rat liver ($t_{1/2} \sim 8$ min)¹⁹, and as a first approximation, suggests that the fractional turnover rate for mixed autophagic vacuole contents is of the order of 0.08 per min.

During cyclic liver perfusion, most amino acids quickly attain steady-state levels that are about the same or only slightly lower than normal plasma values, and a few (including leucine, isoleucine, valine, glutamic acid and glycine) continue to rise¹³. But, as there is no external supply to replace those that are metabolised, a moderate deficiency does in fact exist and extra amino acids are provided by a significant increase in the rate of cytoplasmic proteolysis. It is evident from Figs 2 and 3 that autophagy and lysosomal enlargement increase in these conditions although to a smaller extent than was observed after more stringent deprivation. Our previous failure to demonstrate typical autophagic alterations during cyclic perfusion¹² can probably be explained by the technique of fixation used. In subsequent work we have shown it to be

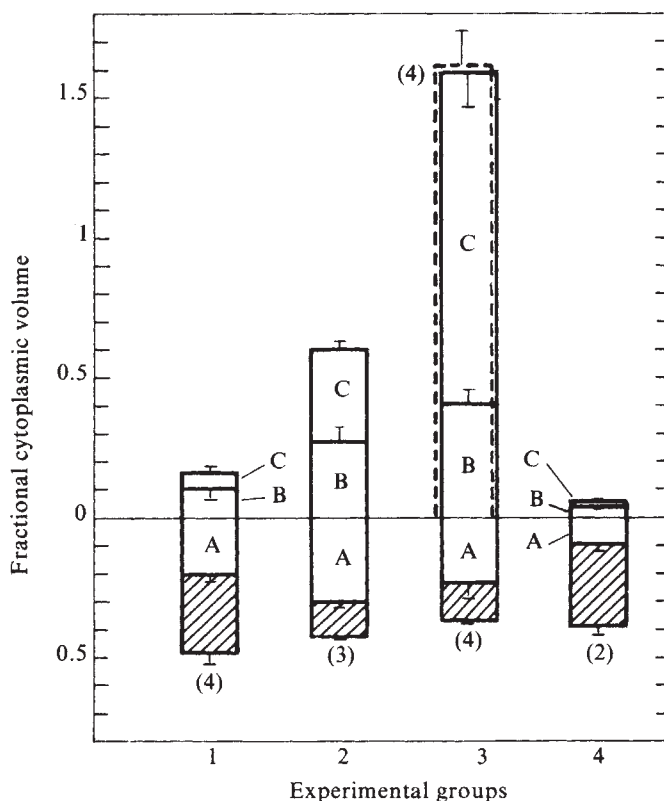


Fig. 2 Fractional cytoplasmic volume of components of the lysosomal-vacuolar system. The following experimental conditions were evaluated: (1), no perfusion; (2), cyclic perfusion for 60 min with no additions of glucose or amino-acids; (3), single-pass perfusion for 40 min with 10 mM glucose but no added amino acids (the offset dashed line represents values obtained from livers perfused 20 min in the same conditions); and (4), single-pass perfusion for 40 min with a medium supplemented with amino acids at a concentration 10 $\times$ normal plasma levels and 10 mM glucose. The components of the lysosomal-vacuolar system have been divided into four groups. Each bar represents mean $\pm$ s.e.m., of hepatic cytoplasm that is occupied by the designated component. The organelles classically described as autophagic vacuoles¹⁻⁸ appear above the horizontal axis. These autophagic vacuoles have been divided into two types: type B contains only elements of smooth endoplasmic reticulum, $\pm$ glycogen; the remainder were classified as type C. A significant proportion of the inclusions within type C were recognisable mitochondria, rough endoplasmic reticulum, or free ribosomes. In addition, type C vacuoles contained unrecognisable membrane remnants and amorphous material which were probably cytoplasmic constituents in various stages of intralysosomal digestion. The remainder of the lysosomal elements, which comprised the population of 'dense bodies' are shown below the horizontal axis of the graph. Many of these elements seemed identical to peribiliary dense bodies generally observed in normal liver tissue^{26,27} (shaded area). Profiles were also observed which had some region of electron-dense material but also contained an area of granularity indicating the presence of glycogen. These profiles were designated type A autophagic vacuoles. Electron micrographs were analysed by the point-counting method²⁸ which involved a test grid of intersecting lines. The method of stratified random sampling²⁹ was used in selecting blocks of tissue from the two major lobes of the liver. Four to six micrographs were examined from each block of tissue. The number of livers in each experimental group is shown in parentheses. For comparison with fractional volumes expressed as % of whole liver volume, the values in this graph may be multiplied by 0.771, the fractional volume of hepatic cytoplasm with respect to the whole liver³⁰.

less effective than the present method in preserving intralysosomal fine structure.

As shown in Fig. 2, lysosomal elements which contained no distinct membrane remnants were divided into two subgroups according to whether they (1) exhibited the usual electron opacity of dense bodies (shaded), or (2), contained additional sharply demarcated zones of electron lucency type A.

Table 1 Liver cyclic AMP levels

Experimental condition	Liver cyclic AMP (nmol per g liver)
Unperfused (9)	0.812 ± 0.080
Perfused (8) no amino acids	0.973 ± 0.184
Perfused (7) 10 × amino acids	0.917 ± 0.171

Liver perfusions were carried out in the single-pass mode for 40 min with or without amino-acids, as described in the text and in Fig. 2 legend. Value are means ± s.e.m. The number of livers from each group is given in parentheses. Livers were rapidly frozen using Wollenberger clamps previously cooled in liquid nitrogen. Total cyclic AMP was determined using either the protein binding assay of Gilman³¹ as modified by Fain *et al.*³² or the procedure of Steiner *et al.*³³.

In all instances, the lucent areas displayed the characteristic granulation pattern of glycogen; these bodies are presumed to be autophagic vacuoles containing glycogen and probably some associated cytoplasmic matrix and smooth endoplasmic reticulum. It is evident from Fig. 2 that the fractional cytoplasmic volume of type A lysosomes tended to increase with amino acid deprivation. It is also apparent from Fig. 2 that the composition of cytoplasmic elements within the larger, more typical vacuoles was not the same in the four conditions

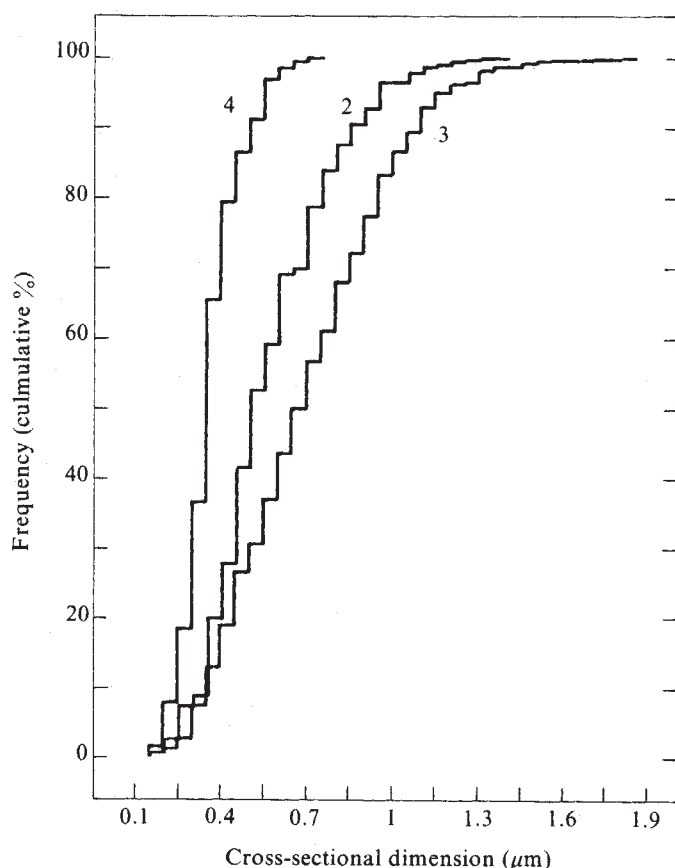


Fig. 3. Cumulative size distribution of dense bodies and autophagic elements (type A, B, and C). The data were compiled from measurements on the micrographs of Fig. 2, and the number on each curve corresponds to the experimental group as described in Fig. 2. Size estimates were obtained by directly measuring the maximum cross-sectional dimensions of profiles on electron micrographs. Dimensions were standardised by correcting for differences in magnification. Dense bodies and autophagic vacuoles were then arranged in size categories to the nearest 0.05 μm. A total of 300 profiles were measured in group 2, 500 in group 3, and 200 in group 4.

studied. The proportion of large forms containing mitochondria and/or fragments of rough endoplasmic reticulum (type C) increased relative to the total autophagic fractional volumes (types B+C) as the level of autophagy increased. Thus sequestration does not seem to be a random process, but exhibits some selectivity according to the degree of amino-acid deprivation.

The effect of amino acid depletion on the sequestration of cytoplasmic constituents by the lysosomal-vacuolar system is seen at normal levels of cyclic AMP and suggests that hepatic autophagy is controlled by amino acids independently of tissue alterations of the cyclic nucleotide. Insulin may also suppress this process¹², but it is clear that maximal inhibition can be obtained by amino acids alone. In view of the magnitude of this autophagic response, it probably could account for most or all of the increase in protein degradation that occurs with amino acid lack. Taking 0.08 per min as a minimal estimate of the fractional rate of turnover of cytoplasmic components (and of protein) within autophagic vacuoles, we have computed that the rate of cytoplasmic processing by type B and C autophagic vacuoles would be 6.0% per h per liver under stringent amino acid deprivation, 2.2% per h with moderate deprivation (cyclic perfusion), and 0.2% per h with no deprivation (amino acid additions). The difference between the last two estimates, 2.0% per h, is capable of accounting for the increase in hepatic proteolysis that occurs in the absence of added amino acids during cyclic perfusion^{12,13}. Since the number of autophagic vacuoles in rat liver had been shown to rise sharply in the postabsorptive period⁷, autophagy could explain much of the loss of total liver protein during this period²⁰. The same process might also account for the enhanced proteolysis observed in cultured eukaryotic cells during nutritional deprivation or in the absence of macromolecular growth substances^{21,23}.

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Monocular astigmatism effects on kitten visual cortex development

SIMULTANEOUS binocular vision during postnatal development is necessary for the maintenance of normal binocular connectivity in the kitten visual cortex. If one eyelid of a kitten is sutured shut throughout early development, three major changes can be observed when the eyelid is opened: (1) cortical cells lose functional connections with the deprived eye and may be activated only by the eye which remained open^{1,2}; (2) cells in the layer of the lateral geniculate nucleus connected to the deprived eye shrink relative to those cells receiving innervation from the normal eye^{3,4}; and (3) tested through its deprived eye, the cat seems to be almost totally blind⁵. We report here investigations on kittens which had been reared in the dark and then given visual exposure wearing goggles. One eye was allowed to view normally and the other looked through a cylindrical lens simulating astigmatism.

The behavioural, physiological and anatomical astigmatism changes which occur in the cat visual system after monocular deprivation are a result of competition between the two eyes, rather than a simple consequence of disuse⁶⁻⁹. Debate now centres on the mechanism of this competition. The two principle hypotheses, as put forward by Guillery⁶ and discussed by others⁷⁻⁹ are first, that cellular changes in the lateral geniculate body are a secondary consequence of a competitive interaction among geniculate terminals at the visual cortex. Here the primary event is the loss of functional connections with the cortical cell. Second, an intrageniculate competition mediated through inhibition between the layers of the geniculate results in a shrinkage of cells connected to the deprived eye. The loss of functional connections with the cortical units is considered to be a secondary consequence of the intrageniculate competition. We have dissociated these hypotheses by rearing kittens with

one eye viewing through a negative (axis vertical) 12-dioptre cylindrical lens whose power exceeds the accommodative range of the cat¹⁰. This lens allows clear vision of horizontal contours but defocuses the image progressively more as the stimulus orientation approaches vertical. As such it simulates the clinical condition of astigmatism¹¹.

Three kittens were reared from birth to 25 d old in the dark. They were then given visual exposure wearing goggles which forced one eye to view through the cylindrical lens while the other viewed normally, for 4–6 h per d for a total of 80–120 h. They were then returned to the dark until they were at least 3-months-old at which point single cell responses were examined in visual cortex. Our procedures for recording responses from single cortical cells are described elsewhere^{12,13}. Kittens were initially anaesthetised with intravenous sodium thiopental and cortical units were recorded extracellularly with glass-coated platinum-iridium microelectrodes. We used the sampling methods of Stryker and Sherk¹⁷, sampling units at intervals of approximately 100 μm in order to minimise the bias of our recorded population. A blind procedure in which the experimenter knew neither the axis of the cylindrical lens, nor which eye wore the negative lens was used throughout the recording sessions. If the competition between the two eyes occurs at the orientation-selective cortical cell, the effect of the lens should be to give the normal eye a competitive advantage at cells preferring vertical stimuli and no competitive advantage at cells preferring horizontal stimuli. The 'cortical' mechanism proposed by Guillery⁶, thus predicts that the effect of the deprivation would be maximal at vertically-orientated cells and minimal at horizontally-orientated cells. If the competition occurs at a more peripheral level, where orientation selectivity among the cells is not present, the 'geniculate' mechanism predicts that the deprivation should reduce the effectiveness of the 'astigmatic' eye equally across cortical units of all orientations.

The distribution of ocular dominance for 292 units encountered in these kittens is shown in Fig. 2. As can be

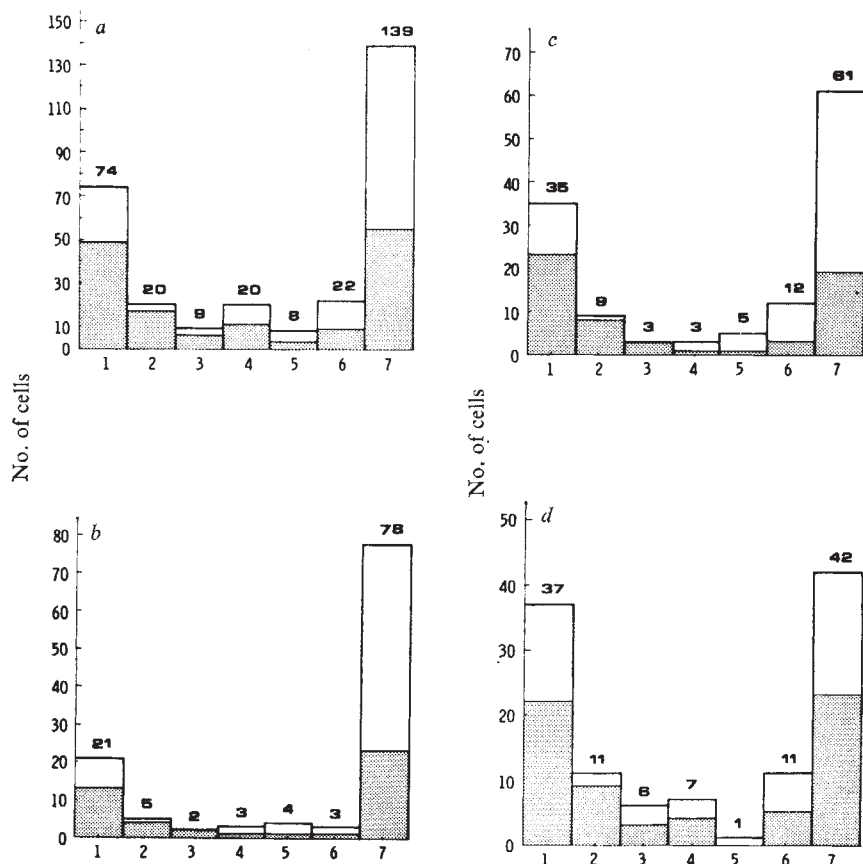


Fig. 1 The distribution of ocular dominance for units in the visual cortex of cats reared with one eye viewing through a cylindrical lens and the other eye viewing normally. The numbers on the abscissa from 1 to 7 represent a trend from the 'astigmatic' eye to the normal eye. Cells in group 1 are driven exclusively by the astigmatic eye; cells in group 4 are driven equally by both eyes; cells in group 7 exclusively by the normal eye. *a*, The distribution of ocular dominance for all cells in these cats. *b*, *c* and *d*, the distribution of ocular dominance as a function of the orientation of the unit. *b*, The vertical and *d*, the horizontal distributions represent cells which prefer orientations within 30° of vertical and horizontal. *c*, The 'diagonal' distribution represents cells preferring stimulus orientations within 15° of either diagonal. The hatched parts of the distribution represent cells in the hemisphere contralateral to the astigmatic eye.

seen, about twice as many units can be influenced through the normal eye as through the astigmatic eye. The distribution of ocular dominance is broken down according to the preferred orientation of the cortical units in the lower parts of Fig. 1. It is clear that the magnitude of the deprivation effect varies depending on the orientation preference of the cortical cell. It is marked for units preferring orientations within 30° of vertical, weaker for diagonally-orientated units and absent for units preferring horizontally-orientated ($\pm 30^\circ$) stimuli. The results thus confirm the cortical hypothesis outlined earlier; they show that binocular competition occurs at the orientation-selective cortical cell and that this competition seems to be sufficient to account for all ocular dominance changes consequent to monocular image blur.

The total number of units encountered which prefer a particular orientation is shown for each eye separately in Fig. 2. The 'astigmatic' eye drives far fewer units which prefer vertically-orientated stimuli than units which prefer horizontal. The histograms in Fig. 2 show that the effect is similar in both hemispheres. One might imagine that this is a simple consequence of this eye having less exposure to focused stimuli with vertical orientations¹⁴⁻¹⁷. Such an explanation cannot, however, account for the striking bias in the distribution of preferred orientations which is evident in the normally-viewing eye. In this eye, 55% more cells prefer stimuli orientated within 30° of vertical than prefer stimuli within 30° of horizontal. This is at first glance surprising, since the normal eye has, after all, viewed all orientations with equal frequency and clarity. An examination of the histograms of Fig. 3 reveals that the distributions in the two eyes are complementary. The trough near vertical for the astigmatic eye is balanced by a corresponding bulge in the normal eye. Combining distributions for the two eyes, the total number of units encountered preferring vertical ($\pm 30^\circ$) and horizontal ($\pm 30^\circ$) is nearly identical. The overall distribution of orientation-selective

neurones in the visual cortex has remained unaltered but the normal eye seems to have made compensatory, orientation-selective inroads into the cortical territory normally occupied by the astigmatic eye.

The effects obtained here are not as marked as those which follow monocular eyelid suture during early development^{1,2}. The latter case differs from ours in that the sutured eyelid almost completely obscures the stimulus while our negative lens merely blurs the image. The observed effects seem more marked in the hemisphere contralateral to the eye which wore the negative lens. This apparent asymmetry is probably attributable to the known preponderance of input from the contralateral eye to the visual cortex¹⁸. The normal contralateral bias would enhance the observed effects in one hemisphere and minimise them in the other.

A puzzle remains in the ocular dominance distribution for horizontally-orientated units where viewing conditions for the two eyes should be equal. While there is no tendency for either eye to control more neurones, the distribution lacks the usual complement of binocular neurones. The most likely explanation for this phenomenon is that the cylindrical lens was not perfectly centred on the kitten's eye. Even a small misalignment between the geometrical centre of the lens and the pupil would lead to prismatic displacement which could vary from day to day. This would mimic the situation in strabismus, a condition which is known to result in a loss of binocularly-driven neurones¹⁹.

Three main conclusions may be drawn from these results: first, asymmetric ocular refractive error during early development can cause severe changes in cortical binocular connectivity. Second, the mechanism of this disruption is a developmental competition between the two eyes which occurs at the visual cortex. And third, the cortical distribution of preferred orientations in a normally-viewing eye can be biased merely by depriving the other eye of focused input at certain orientations.

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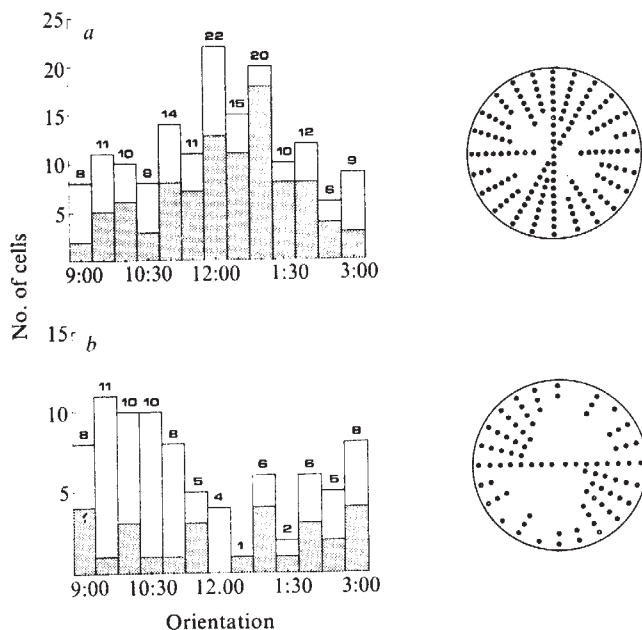


Fig. 2 Distribution of preferred orientations for cortical units driven by *a*, the normal and *b*, the astigmatic eyes. On the right are polar plots in which each cell represents a single dot. The length of the dotted line along a given orientation is thus proportional to the number of units encountered which have this preferred orientation. On the left, the same data are presented in histogram form and are further subdivided by cortical hemisphere. The height of the bar at any orientation is proportional to the number of units preferring that orientation. The hatched part of the distribution is derived from the hemisphere contralateral to the astigmatic eye. The horizontal cells of the polar plots are divided equally between 9:00 and 3:00 on the associated histograms.

Diffusion barrier for acetylcholine at the frog neuromuscular junction

INACTIVATION of acetylcholinesterase (AChE) causes the half-decay times of the endplate currents (e.p.cs) and miniature e.p.cs (m.e.p.cs) to increase from about 1.5-2.0 ms to 3.0-10.00 ms, times far too long to be accounted for by removal of the transmitter by free diffusion¹⁻³. To explain this, Katz and Miledi¹ postulated

that, when the AChE is blocked, each acetylcholine (ACh) molecule undergoes numerous collisions with the post-synaptic membrane, leading to several attachments with the ACh receptors and a consequent delay in transmitter removal. This was supported by the observation that (+)-tubocurarine, which reduces the number of free receptors, markedly decreases the half-decay of m.e.p.s and e.p.s. after treatment with neostigmine^{1,3}. In addition to this delaying mechanism, due to interaction with the ACh receptors the results described below suggest the existence of a second diffusion barrier for the transmitter at the frog neuromuscular junction. This barrier seems to be formed by non-depolarising sites which bind the ester moiety of ACh. Saturation of these sites with substances which possess $-\text{CO}-\text{O}-$ or $-\text{CO}-\text{O}-\text{CO}-$ groups accelerates the diffusion of externally applied ACh towards the ACh receptor, enhancing its depolarising effects. The barrier also hinders the outward diffusion of neurally released ACh in neostigmine-treated muscles, but this effect can only be demonstrated if the motor nerve is stimulated at high frequencies (200 Hz).

Compounds which possess an ester or carboxylic anhydride group, such as methyl and ethyl acetates, butyrolactone and succinic anhydride (SA), activate the ACh receptor. In addition, at concentrations just enough to cause a depolarisation of a few millivolts, these compounds markedly potentiate the effect of externally applied ACh^{4,5}. The mechanism of this potentiating action was investigated using SA. Although this compound undergoes spontaneous hydrolysis at a relatively high rate⁶, its activity in Ringer's solution (pH 7.2) is maintained over sufficiently long experimental periods and does not adversely affect the muscle fibres.

Using the moving electrode technique described by Fatt⁷, we observed that the presence of SA in the bath increased the depolarising action of ACh on the frog sartorius muscle by an average factor of 1.5 ($n=11$; range 1.3–1.8). One of the experiments is illustrated in Fig. 1a. Analogous results were obtained with carbamylcholine and also with ACh in muscles treated with neostigmine. This excluded the possibility that the increased depolarisations were due

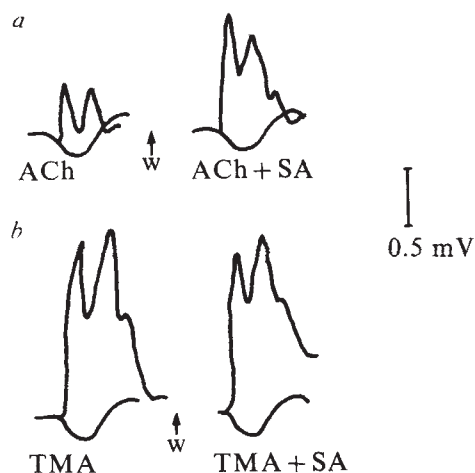


Fig. 1 Effect of SA on the depolarising action of ACh and TMA on frog (*Rana pipiens*) sartorius muscle. *a*, Depolarisation produced by 10 μM ACh in the absence or presence of 1 mM SA. *b*, Obtained from the same muscle in response to 100 μM TMA before and after addition of 1 mM SA. Arrows indicate periods of washing with normal Ringer's to re-establish the original baseline profile (the bottom tracing in all records). The indicated concentrations of ACh and SA were used throughout at 21°C (except in one of the experiments, Table 1) dissolved in a Ringer's solution with the following ionic composition (mM): Na 113.37, K 1.96, Ca 1.76, all as chloride salts. TES 5 mM was used to keep the pH at 7.2. Neostigmine 3 μM was also present in the solution as indicated. Muscles were kept in neostigmine for at least 30 min before recording.

to blockade of AChE. Similarly, measurements of the input resistance of the muscle fibres, with and without SA in the bath, failed to reveal any effect of this compound on the resistance of the muscle membrane, which, if increased, could also explain the enhanced depolarisation.

In spite of its potentiating effect on bath and electrophoretically applied ACh, SA did not affect the average amplitude of the miniature endplate potentials (m.e.p.s). The mean size of the m.e.p.s recorded from four fibres was of 0.57 ± 0.1 mV (s.e.m., $n=290$) in normal Ringer's and of 0.59 ± 0.06 mV (s.e.m., $n=423$) in the presence of SA. Since it has been shown that the receptors which interact with applied ACh are identical to those activated by the intrinsically released transmitter⁸, this result indicates that SA does not exert its effect at the level of the ACh receptors. On this basis, we hypothesised that SA removed a barrier to the diffusion of ACh from the external solution towards the ACh receptors, thus allowing a higher effective concentration of the agonist at the receptor level. The barrier is not likely to be structural, since the effects of SA were reversed by washing the preparation. Thus, SA most probably saturates non-depolarising receptors to which ACh normally binds with high affinity. Since SA lacks a quaternary ammonium group, a site with affinity for both ACh and SA must involve the common $-\text{CO}-\text{O}-$ group. This leads to two predictions: first, SA should not potentiate the effects of nicotinic agonists which lack $-\text{CO}-\text{O}-$ groups; and second, SA should accelerate the diffusion of ACh both to and from the synaptic cleft.

The first prediction was easily confirmed. SA did not potentiate the depolarising action of either tetramethylammonium (TMA) or nicotine. One of these experiments is illustrated in Fig. 1b. The records, obtained from the same muscle as those in Fig. 1a, show the depolarisation elicited by TMA before and after the addition of SA. The lack of potentiation cannot be attributed to a saturation of the response, since larger depolarisations were elicited afterwards in the same preparation.

Changes in the rate of outward diffusion of ACh were studied measuring the time course of e.p.s. in muscles treated with neostigmine, in the presence and absence of SA. These measurements were carried out with a two microelectrode voltage clamp system⁹, and the potential across the endplate membrane was fixed at 100 mV (inside negative) throughout the experiments. The excitation-contraction mechanism was previously uncoupled by immersing the muscles in a 1.5 or 2.0 M solution of formamide¹⁰.

No effect of SA on the time course of single e.p.s. was observed (Fig. 2). This indicates that the prolonged decay of these e.p.s. in the presence of neostigmine can be attributed only to delayed diffusion caused by multiple receptor interactions. When the synaptic space is 'overloaded' with neurally released transmitter, however, it is possible to show an action of SA on the e.p.s. In neostigmine-treated muscles, the half-decay time of an e.p.c. is slower if evoked 5 ms after another³. Accordingly, when we applied a few stimuli to the motor nerve at 200 Hz, the half-decay time of the last e.p.c. of each volley increased progressively with the number of motor impulses. This reflects the fact that the concentration of ACh in the synaptic space increases with each impulse at a rate faster than it is lowered by diffusion alone.

The open circles in Fig. 2 illustrate the increased average half-decay time of the e.p.s. after four successive stimuli. The filled circles show the average half-decay times obtained in the same fibres in the presence of SA. In both experimental conditions the half-decay times increased; however, in the presence of SA this increase is smaller. In the fourth e.p.c. the half-decay time is significantly lower ($P < 0.025$) than that in the absence of SA. Such observations support the view that SA increases the

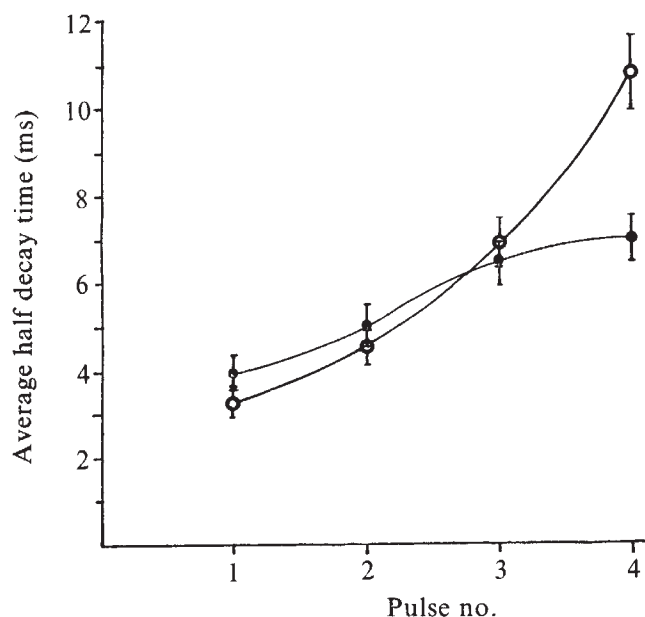


Fig. 2 Average half-decay times of e.p.c.s expressed as a function of the stimulation sequence. ○, Average half-decay times of e.p.c.s evoked by four successive pulses in a total of 10 fibres in the presence of 3 μ M neostigmine. ●, Same measurements obtained from the same fibres when 1 mM SA was added to the bath solution (note tendency to reach a steady level). Vertical bars represent s.e.m. Intervals between stimuli, 5 ms.

rate of flow of ACh across the diffusional barrier also in an outward direction.

The existence of non-depolarising binding sites for ACh in the neuromuscular junction has already been considered by Katz and Thesleff¹¹. These authors observed a "more than linear" summation of the depolarisations produced by two small doses of ACh applied simultaneously to the same site. They suggested that a large portion of the molecules in a single dose might occupy binding groups with high affinity for ACh but with no depolarising power. When an additional dose is applied, a smaller fraction would be retained at these sites and more ACh molecules would be available for depolarisation.

We investigated the effect of SA on the summation of small depolarisations elicited simultaneously by ejecting ACh electrophoretically from the barrels of a twin micropipette. Table 1 summarises results from two muscles immersed first in normal Ringer's and then in either 1 or 2 mM SA. As Katz and Thesleff have reported¹¹, the amplitude of the ACh potentials superimposed on a steady

depolarisation is often larger than that of the control ACh potentials. The magnitude of this potentiation averaged 1.54 times in normal Ringer's. As can be seen, SA blocks the occurrence of such potentiation. These results suggest that the non-linear initial region of the dose-response curve reflects primarily the retention of ACh molecules by the SA-sensitive barrier.

The physiological role of a diffusion barrier between the synaptic cleft and the extracellular solution is open to speculation. Such a barrier may retain ACh within the postsynaptic compartment but still removed from the receptors. Thus, the loss of ACh molecules would be minimised and more substrate would be available for the choline recycling mechanism. Regardless of any possible physiological significance, the presence of this barrier must be taken into account in pharmacological experiments designed to evaluate the action of agonists on the endplate receptors.

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A simple and sensitive radioreceptor assay for antischizophrenic drugs in blood

THE neuroleptics, comprised primarily of phenothiazines, butyrophenones and thioxanthenes, represent the major drugs used in treating schizophrenic symptoms. For several reasons, routine monitoring of patient blood levels of neuroleptics would be desirable. The dose requirement for therapeutic response varies markedly, related in major part to a wide range in blood levels¹⁻⁴. Because the severe motor side effect of tardive dyskinesia⁵ may be very long-lasting and is apparently dose related, patients should be maintained on the lowest dose required for therapeutic efficacy^{5,6}. While blood levels must exceed a threshold to elicit symptomatic improvement, too high a blood level may reduce the therapeutic response⁷⁻⁹. The available assays for plasma neuroleptics, including gas chromatography, fluorimetry, formation of radiolabelled derivatives^{4,10-14} and radioimmunoassay^{15,16} have not attained routine clinical use because of technical complexities or restrictions of specificity to single drugs. We describe here an assay for neuroleptics based on competition for dopamine receptor binding which is simple, sensitive, specific and suitable for routine clinical application.

Neuroleptics in clinical use are thought to exert their therapeutic antischizophrenic actions by blocking brain dopamine receptors¹⁷⁻¹⁹. The radiolabelled butyrophenones, ³H-haloperidol and ³H-spiroperidol, bind selectively and with high affinity to dopamine receptor sites in mammalian brain; this specific binding is reduced by neuroleptics in close parallel with their clinical potencies¹⁹⁻²². Most neuro-

Table 1 Average maximum amplitudes of ACh potentials relative to their constant, 'pre-steady depolarisation', values

Experiment	AChP _{max} : AChP _{const}		
	Normal	SA 1 mM	SA 2 mM
1	1.56 ± 0.33 (11)	1.08 ± 0.20 (18)	
2	1.52 ± 0.35 (19)		0.97 ± 0.33 (26)

AChP_{max}, amplitudes of largest ACh potential obtained during the steady depolarisation AChP_{const}, pre-depolarisation amplitude of the constant ACh potential. Relative amplitudes are expressed as averages of maximum/constant ratios ± s.d. Numbers in parenthesis indicate number of measurements. ACh potentials were obtained applying current pulses (0.5-1 Hz) to one barrel, adjusted until potentials of a constant amplitude (2-5 mV) were produced. A steady depolarisation of 3 mV was then imposed by a small direct current flowing through the second barrel.

leptics inhibit ^3H -haloperidol and ^3H -spiroperidol binding in concentrations substantially lower than those which occur in the blood of patients at therapeutic doses. The radio-receptor assay described here is based on the principle that neuroleptics in the patient's serum compete for the binding of ^3H -haloperidol or ^3H -spiroperidol to dopamine receptors in membranes of the corpus striatum (S. H. S., unpublished).

In this assay, serum is used without any extraction or other purification procedure. The absence of interference by serum is apparent from experiments in which 15 μl of serum in a total assay volume of 1 ml reduced ^3H -haloperidol or ^3H -spiroperidol binding by only 10–15%. Increasing volumes of serum reduced binding linearly with about 30% inhibition occurring with 150 μl of serum. Small volumes of serum reduce both specific and non-specific ^3H -ligand binding to similar extents suggesting that serum proteins bind ^3H -ligand, making less available for interactions with brain membranes. The degree of inhibition of ^3H -ligand binding by control sera from several laboratory personnel was uniform. Specific binding of ^3H -haloperidol in the presence of 15 μl of serum from 18 different subjects was $91 \pm 1.6\%$ (total binding 2,325 c.p.m. blank with 0.1 mM dopamine 953 c.p.m.) of control values while specific ^3H -spiroperidol binding was $85 \pm 1.2\%$ (total binding 1,264 c.p.m. blank with 1 mM dopamine 191 c.p.m.) of control for 10 different subjects. This small degree of variability in inhibition of ^3H -ligand binding by control sera falls within the error for repeated determinations of the same sample.

Neuroleptic present in a serum sample reduces ^3H -ligand binding beyond the small reduction attributable to serum alone and the degree of inhibition of specific ^3H -ligand binding is proportional to the amount of neuroleptic present. By constructing a standard curve of inhibition of specific ^3H -ligand binding by known amounts of the drug, the amount present in a serum sample can be easily determined.

This method requires that the presence or absence of neuroleptic in a serum sample will not affect the degree of nonspecific (blank) ^3H -ligand binding. In 46 serum samples from patients on neuroleptics, blank ^3H -haloperidol binding was 887 ± 6 c.p.m. while nonspecific binding in the presence of five control sera was 894 ± 17 c.p.m. In six patients on haloperidol, nonspecific ^3H -spiroperidol binding was 199 ± 4 c.p.m. and 198 ± 6 c.p.m. in the presence of six control serum samples. Thus specific binding in the presence of patient sera can be determined by subtracting nonspecific binding values obtained with control sera from total binding with patient sera. The percentage inhibition of specific ^3H -ligand binding (in the presence of control serum) by the patient serum (containing drug) is then calculated and compared to a standard displacement curve for determining actual neuroleptic content.

Up to 99% of neuroleptics may be bound to blood components^{23,24}. In this assay the neuroleptic bound to blood proteins dissociates during the incubation so that total neuroleptic levels are measured. In confirmation of this assumption in one experiment 15 nM haloperidol was preincubated for 10 min at 37 °C with control serum to allow binding to serum proteins to occur. The serum and haloperidol was then added to a standard binding assay which was at equilibrium and it progressively reduced ^3H -haloperidol binding with increasing durations of incubation, with a maximal lowering by 5–10 min. The time course and extent of reduction of binding was the same for haloperidol pre-incubated with serum or simply dissolved in water. The maximum percentage lowering of binding was equivalent to that produced by 15 nM haloperidol added directly to the assay with no serum present.

For the neuroleptic radioreceptor assay fresh rat striatum was sonicated in 100 volumes (w/v) 50 mM Tris buffer, pH 7.7 at 25 °C with a Sonifier, setting 4 for 30 s. The homogenate was centrifuged twice at 50,000g for 10 min (Sorvall RC2-B) with rehomogenisation of the intermediate pellet in fresh buffer. The final pellet (which may be stored frozen) was resuspended in 125 volumes, for assays using ^3H -haloperidol, or 285 volumes for ^3H -spiroperidol, of freshly prepared 50 mM Tris buffer containing 0.1% ascorbic acid, 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 and 1 mM MgCl_2 to give a final pH of 7.1 at 37 °C. ^3H -Haloperidol (15 Ci mmol⁻¹, New England Nuclear) or ^3H -spiroperidol (26 Ci mmol⁻¹, New England or Amersham) was diluted to 10 nM or 1 nM, respectively, in fresh 0.1% ascorbic acid. Glass 12 $\times$ 75 mm incubation tubes received, in order, 15–150 μl serum (on drug therapy or drug free) 100 μl ^3H -ligand, 100 μl drug for standard curve or dopamine for blanks (10^{-4} M for ^3H -haloperidol and 10^{-3} M for ^3H -spiroperidol) or the drug solvent 0.1% ascorbic acid, and tissue suspension to 1 ml total volume. Final concentrations of ^3H -haloperidol or ^3H -spiroperidol were 1 nM and 0.1 nM respectively. The tubes were incubated at 37 °C for 10 min (^3H -haloperidol) or 15 min (^3H -spiroperidol) and rapidly filtered under vacuum through Whatman GF/B filters with three 5-ml rinses of ice-cold 50 mM Tris buffer, pH 7.7 at 25 °C. ^3H -Ligand trapped on the filters was counted by liquid scintillation spectrometry after remaining overnight in scintillation vials containing NEN formula 947 fluor (New England Nuclear). A standard displacement curve for the drug under study was determined in the presence of equal volumes of control serum with final concentrations of the drug about one-third, three times and the same as its K_i value determined previously^{19,22}. A log-probit plot was used to convert the displacement curve to a straight line so percentage inhibition of ^3H -ligand binding can be easily converted to molar drug concentration (Fig. 1). In the presence of serum the ratio of total to nonspecific binding was about 2.4 for ^3H -haloperidol and 6.6 for ^3H -spiroperidol, so that the latter is the preferred ligand. Plasma can be used for assays if the KCl, CaCl_2 and MgCl_2 in the buffer are replaced by 5 mM sodium EDTA which prevents the plasma from clotting the brain homogenate. Neuroleptics can also be eluted from red blood cells by sonification and incubation in 0.1% ascorbic acid.

To examine recovery five neuroleptics were preincubated with serum for 10 min at 37 °C, to provide time for binding to serum proteins and subsequently assayed for

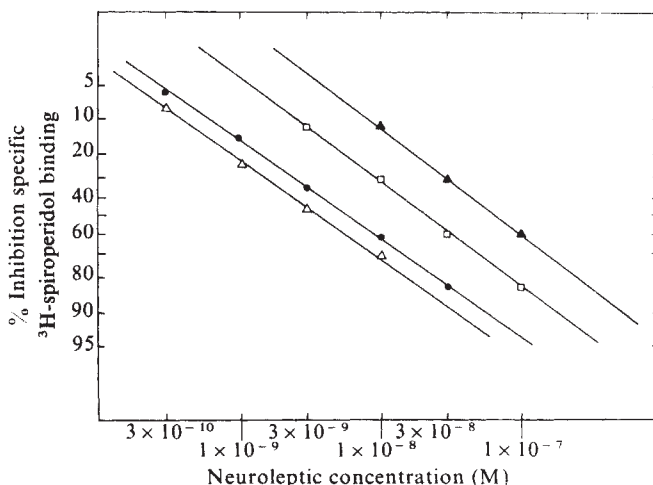


Fig. 1 Log-probit plots of neuroleptic inhibition of specific ^3H -spiroperidol binding to rat striatal membranes. $\blacktriangle$, Thioridazine; $\square$, chlorpromazine; $\bullet$, haloperidol; $\triangle$, fluphenazine.

neuroleptic levels. Haloperidol (0.1 μ M), fluphenazine (0.1 μ M), trifluoperazine (0.1 μ M), chlorpromazine (1 μ M), and thioridazine (1 μ M) were all fully recovered in the neuroleptic radioreceptor assay with respective values of $0.11 \pm 0.02 \mu$ M ($n=6$), $0.12 \pm 0.01 \mu$ M ($n=10$), $0.16 \pm 0.2 \mu$ M ($n=6$), $1.0 \pm 0.1 \mu$ M ($n=9$) and $1.1 \pm 0.2 \mu$ M ($n=6$). To examine the recovery of neuroleptics from the blood of patients treated *in vivo* we measured blood levels in four patients receiving oral doses of haloperidol both after extraction into organic solvent (heptane-5% isoamyl-alcohol, back extracted into 0.1% ascorbic acid) which should remove all drug bound to serum protein, and by adding serum directly to the binding assay as in the above method. Serum drug levels ranged between 10 and 250 nM and agreed within a mean of 10% whether assays were conducted with or without extraction.

To assess the validity of the radioreceptor assay, we compared its results with blood levels measured by other procedures. In one study, rats were given 3 H-haloperidol 0.2 mg per kg body weight and blood levels of the drug were assessed by determining plasma radioactivity 10 min after drug administration or by radioreceptor assay following organic solvent extraction of the plasma. At this interval after 3 H-haloperidol administration at least 75% of the radioactivity in plasma can be accounted for as unmetabolised haloperidol²⁵. The blood level was 130 ± 30 nM by measuring radioactivity and 120 ± 30 nM by radioreceptor assay. In other studies, we measured blood haloperidol levels in patients whose levels were also determined by radioimmunoassay and found a good agreement between the two procedures (Table 1) (I.C., R. J. Wyatt and S.H.S., in preparation). Serum samples have been assayed fresh or after storage at or below -20°C , but repeated freezing and thawing led to reduced measured neuroleptic levels. Using 150 μ l of serum the lower limit of sensitivity (15% inhibition of specific binding) is 1.8 ng ml⁻¹, 2.2 ng ml⁻¹, 2.5 ng ml⁻¹, 8.6 ng ml⁻¹ and 30 ng ml⁻¹ for fluphenazine, trifluoperazine, haloperidol, chlorpromazine and thioridazine, respectively.

Table 1 Comparison of radioreceptor assay (RRA) and radioimmunoassay (RIA) values for plasma levels of haloperidol

Patient samples	Dose level, Haldol (mg d ⁻¹)	Haloperidol (ng ml ⁻¹)	
		RRA	RIA
1	20	27	27
2	20	29	28
3	10	21	18
4	20	31	37
5	10	22	20
6	20	29	28

Aliquots of 30 μ l of patient plasma samples (previously frozen at -20°C) were assayed in the radioreceptor assay with 3 H-haloperidol as ligand. A standard curve of 3 H-haloperidol displacement was constructed from known amounts of haloperidol in the presence of control plasma. The radioimmunoassay²⁹ for haloperidol was performed by Drs Michael Shostak and James Perel, Columbia University.

In summary, this radioreceptor assay seems to offer a potential for routine application to large populations of patients. The procedure is sensitive. Since the milligram potencies of neuroleptics are closely correlated with their affinity for dopamine receptors labelled with 3 H-haloperidol^{19,21}, the sensitivity of the assay is greater for drugs which are used therapeutically at lower doses. At therapeutic doses of many neuroleptics, we have found the radioreceptor assay sensitive enough to determine drug levels in 15–30 μ l serum samples. But, where steady state blood levels are relatively low the sensitivity of the assay can be increased up to 10-fold by increasing the volume of serum (up to 150 μ l) added to the assay. The assay is selective for neuroleptics. Only dopamine receptor

antagonists compete with high affinity for 3 H-haloperidol and 3 H-spiroperidol binding. Levels of circulating catecholamines are much too low to pose any problem. In screens of large numbers of drugs from different chemical classes no drugs other than neuroleptics, except for ergots, compete significantly for 3 H-haloperidol binding²⁶. Ergots which do bind to dopamine receptors are not usually used clinically in the therapy of schizophrenics. Thus, this assay can be used in patients who are also receiving a variety of other drugs besides neuroleptics. For patients treated with two or more different neuroleptics, the absolute concentration of each drug cannot be separately determined. In these cases the total inhibition of 3 H-haloperidol or 3 H-spiroperidol binding can be converted to a 'chlorpromazine equivalent' which would produce the same degree of inhibition.

Another virtue of the assay is that it will detect metabolites which compete for dopamine receptor binding and thus may be therapeutically active. There is evidence that a substantial portion of the therapeutic activity of chlorpromazine might be contributed by its metabolite 7-hydroxychlorpromazine²⁷. 7-Hydroxychlorpromazine has about 70% of the affinity of chlorpromazine itself for 3 H-haloperidol binding sites²⁸ and hence would be detected in this assay.

The radioreceptor assay described here is simple to perform. Substantial quantities of corpus striatum tissue can be obtained from large animals such as calf or pig and stored frozen. 3 H-Haloperidol and 3 H-spiroperidol are commercially available. As many as 100 assays can be conducted in a morning.

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Specific localisation of neurotensin to the N cell in human intestine by radioimmunoassay and immunocytochemistry

NEUROTENSIN, originally isolated from bovine hypothalamus¹, was shown to be a peptide of 13 amino acids². It has been synthesised³ and its pharmacological actions shown to include effects on small intestine motility, blood vessel tone, enhancement of glycogenolysis, release of glucagon and inhibition of insulin release⁴⁻⁷. Immunoreactive neurotensin has been detected in mammalian hypothalamus and a few other areas of the brain⁸⁻¹⁰ but much larger quantities occur in the intestine¹¹. Neurotensin has been shown in discrete cells in the ileal mucosa of various birds and mammals including man¹²⁻¹⁵. The cell type has recently been fully characterised and its identity internationally accepted (see ref. 16). The distribution of the peptide in man is unknown. Using a combination of radioimmunoassay (RIA) and immunocytochemistry (IC) we have found large amounts of neurotensin-like reactivity in human ileum with less in the jejunum. The immunoreactive cells are restricted to the mucosa. Neurotensin thus joins the growing group of peptides which are common to brain and gut¹⁷. The effect of such peptides may differ according to their location. Neurotensin has been reported in rat blood¹¹ and may therefore act as a circulating hormone. In addition, its location in the brain suggests a possible role as a peptidergic neurotransmitter.

Fresh samples of human gastrointestinal tract were obtained during surgery for various disease states and were later found to be histologically normal. These included body and antrum of stomach, duodenum, jejunum, ileum, colon, pancreas and gall bladder. Part of the material was fixed in *p*-benzoquinone vapour¹⁸ and part in purified glutaraldehyde¹⁹ for ultrastructural studies. Pairs of serial 1- μ m resin-embedded sections were treated by an indirect immunocytochemical procedure with antisera to neurotensin and to one of the following peptides: somatostatin, glucagon, vasoactive intestinal peptide (VIP), gastrin, gastric inhibitory peptide (GIP) and cholecystokinin (CCK). The controls used included previous absorption of the antisera with glucagon, somatostatin, VIP, CCK, gastrin, GIP and neurotensin, at concentrations ranging from 2 to 25 μ g per ml of diluted antisera. Negative staining with anti-neurotensin was obtained only after inactivation with at least 10 μ g of synthetic neurotensin per ml of diluted antibody. Negative staining was also obtained by the use of normal rabbit serum instead of immune serum.

For radioimmunoassay a part of each surgical sample was extracted by boiling for 5 min in distilled water and two homogenisations. The tissue was then further extracted in 0.1 M formic acid. Both water and acid extracts were assayed at 10-fold serial dilutions. Antibodies for immunocytochemistry and radioimmunoassay were raised in rabbits to synthetic neurotensin coupled by carbodiimide to bovine serum albumin and by glutaraldehyde to bovine thyroglobulin. ¹²⁵I-Neurotensin was prepared by chloramine T oxidation and was stable at -20 °C for several months. The assay was sensitive to 10 pmol per tube and showed no cross reaction with any other gut hormone. Antibody interference by nonspecific effects was negligible, but was anyway unlikely because the assay sensitivity necessitated assay of extracts only in considerable dilution.

Many endocrine cells, stained solely with antibodies to neurotensin, were located in a restricted area of the gut, being especially numerous in the ileal mucosa. Their number diminished as the jejunum was reached and they were extremely sparse in the duodenum. Neurotensin immunoreactive cells were not detected in the mucosa of

stomach, pancreas or colon, or in any structure in the other layers of the gut wall. They were situated in the upper two-thirds of the villi (Fig. 1a) and were often seen to communicate with the intestinal lumen by means of microvilli (Fig. 1b). The cells were oval in shape and their secretory granules, located mainly in the basal parts of the cell (Fig. 1b), had an average size of 300 nm and a closely attached membrane (Fig. 1c). The neurotensin immunoreactive cells were not members of the argentaffin subgroup of APUD cells²⁰ since they were unstained with the Masson silver impregnation technique.

When serial 1- μ m sections were stained with antibodies reacting with enteroglucagon (EG) and VIP the positive cells were completely distinct from those stained by the neurotensin antibodies. The EG-positive cells contained

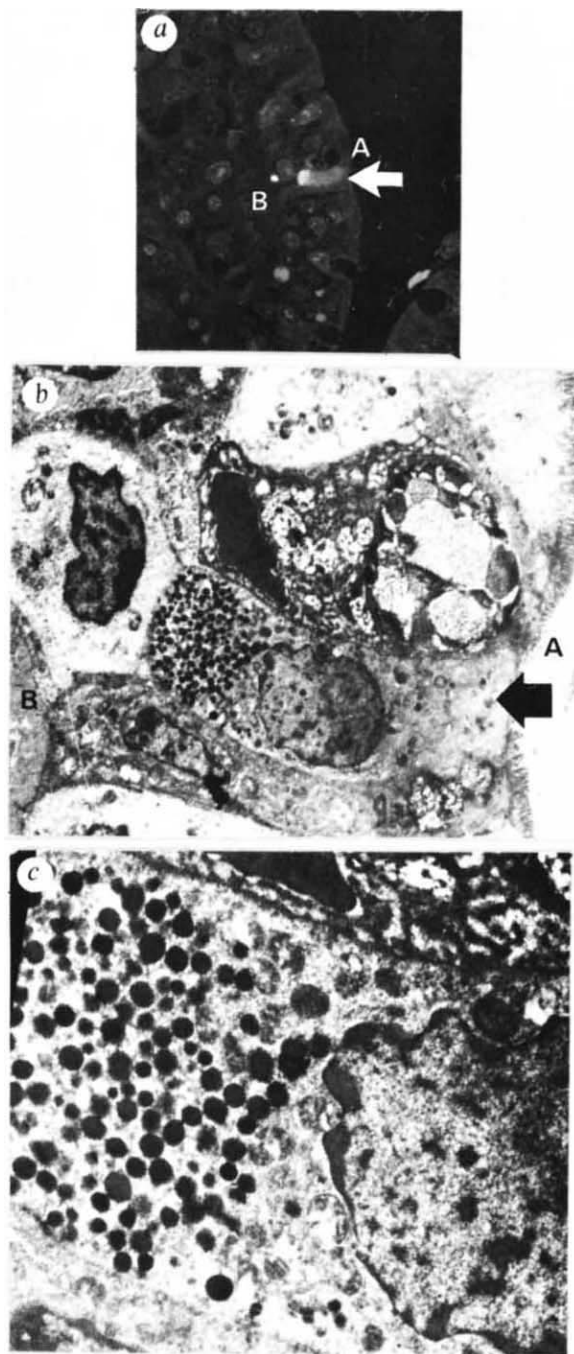


Fig. 1a, 1- μ m Araldite section stained for neurotensin. Arrow marks positive cell. A, gut lumen; B, lamina propria. $\times 625$. b, Serial 40-nm section showing same cell at EM. Arrow marks cell. A, gut lumen; B, lamina propria. $\times 4,000$. c, High magnification of cell to show detail of secretory granules. $\times 14,000$.

round granules with a mean diameter of approximately 260 nm and a closely attached membrane, which thus were considerably smaller than those of the N cell. The VIP-positive cells varied in shape and contained much smaller granules with a mean diameter of 120 nm and a closely attached membrane.

Thus neurotensin is confined to a single type of endocrine cell which has previously been overlooked in ultrastructural studies because of confusion with the EG(L) cells²¹. With the application of electron immunocytochemical methods it can now be seen that the cell contains granules of a type distinct from those of the other endocrine cells in the ileal mucosa.

Neurotensin immunoreactivity was detected by radioimmunoassay in high concentration in the ileal mucosa with lesser quantities in the jejunal mucosa (Table 1). Very much smaller quantities were found in whole thickness extracts of the wall of the stomach, duodenum and colon. It was undetectable in the pancreas and gall bladder.

Table 1 Neurotensin immunoreactivity in various tissues

	Neurotensin (pmol per g wet weight)
Stomach (6)* full thickness	0.27±0.03
Duodenum (7) full thickness	0.24±0.10
Jejunum full thickness (2)	0.80 (1.19, 0.42)
mucosa (3)	2.82 (4.62, 1.78, 2.07)
Ileum full thickness (2)	12.0 (7.57, 16.4)
mucosa (3)	16.2 (23.1, 19.0, 6.5)
Colon (4)	0.50±0.19
Gall bladder (3)	< 0.03
Pancreas (2)	< 0.03

Values are means ± s.e.m.

*No. of determinations.

Neurotensin was originally extracted from the hypothalamus¹, and its function there is no more clear than in the gastrointestinal tract. The morphological features of the neurotensin cells, which have microvilli and secretory granules located at the vascular pole, suggest they are sensitive to changes in the gut lumen which may thus provide the stimulus for the release of the peptide, presumably, directly into the circulation. This view is supported by the finding of neurotensin in rat plasma¹¹. The restricted localisation of the peptide suggests that it may play a precise part in the sequence of post-digestive processes.

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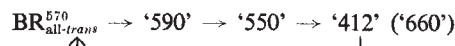
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Hydration effects on the photocycle of bacteriorhodopsin in thin layers of purple membrane

The purple membrane of *Halobacterium halobium* acts as a light-driven proton pump, producing a transmembrane proton gradient which is coupled to ATP synthesis¹, and to phototaxis² in the intact bacteria. It contains a single type of protein, bacteriorhodopsin (BR) which spans a 45-Å membrane. The isolated purple membranes are flat oval sheets with an average diameter of 0.5 µm (refs 3, 4). Bacteriorhodopsin contains a retinal molecule (all-*trans* and 13-*cis*)⁵ which is covalently bound via a protonated Schiff base to a lysine residue. It undergoes a photocycle described by the following scheme^{6–8}:



where proton ejection to the bulk solution occurs on the route '550' → '412' (refs 9, 10), whereas protonation of the bacteriorhodopsin takes place parallel to the '412' → BR_{all-trans}⁵⁷⁰ process¹¹. It has been reported that the reconstituted BR_{13-cis}⁵⁴⁸ undergoes a cycle which involves the 'X' and the '610' intermediates¹². It was demonstrated that proton transfer is a vectorial process where the proton is ejected from one side of the purple membrane and reprotonation takes place on the other side¹³. We present here results on the effects of the specific hydration of the purple membrane on the relaxation times of '412' and on the formation of the '660' and '610' intermediates. The results demonstrate that the full photocycle of bacteriorhodopsin can be observed in thin purple membrane layers even at the lowest hydration state and that the amount of absorbed water is rate limiting for the molecular process of the cycle.

Purple membranes were isolated from *H. halobium* (mutant NRL R₁M₁)⁶. Thin layers of purple membrane were prepared by drying concentrated suspension (3.5 × 10⁻⁴ M) of the purple membrane in water (pH 7.2), on a glass slide. The drying was performed at atmospheric pressure, 45% relative humidity and 25 °C. The average thickness of the preparations was 1–3 µm as determined by scanning electron microscopy. Variable degrees of hydration of the purple membrane were obtained by equilibrating the samples with different relative air humidities produced by saturated salt solutions¹⁴. The glass slide with the preparations was inserted in a 1 × 1 cm cuvette and incubated in a desiccator at the required specific humidity for about 24 h. Before measurement the cuvette was immediately sealed within the desiccator with a Teflon plus Parafilm cover. The limit of dryness, obtained by drying the membrane at 10⁻³ torr for approximately 4 h, was defined as the 0% relative humidity state.

Flash photolysis spectroscopy of thin-layer preparations of purple membrane show that bacteriorhodopsin undergoes a complete photocycle, involving the same intermediates characteristic of suspended purple membrane fragments in water. But the decay time of the '412' intermediate as well as the formation of '660' and '610' intermediates are determined by the specific hydration state of the membrane. Preparations equilibrated with 94% relative humidity show the same kinetics and

include the same transients as in purple membrane fragments suspended in water (Table 1). The relaxation times for '412' decay are slowed down by lowering the hydration state of the preparation (Fig. 1). The kinetics of '412' decay are analysed in terms of the sum of two or three exponentials (Fig. 2). The relaxation times and their relative amplitudes, at different hydration states, are given in Table 2. The initial values for the relaxation times and their corresponding amplitudes were obtained first graphically and then were optimised by a least square program (Harwell Subroutine Library, VCO5A). Essentially the same results were obtained when the initial values were calculated by a computer program (K. H. Mueller and T. Plesser, unpublished), within the frame of a non linear approximation^{15,16}. The thermal decay kinetics of '412' could be enhanced by a factor of 15 when photobleached with 412-nm light at 0% relative humidity, in accordance with earlier experiments carried out with suspended purple membrane¹⁷.

The '660' and '610' intermediates are not formed at relative humidity lower than 90%. Moreover, the 660-nm intermediate cannot be detected at pH higher than 8 (in a solution of 4M

Table 1 Relaxation time constants (τ_i) of the '412', '660' and '610' intermediates

Intermediate	Purple membrane in water* (τ , ms)	Thin purple membrane layers† (τ , ms)
'412'	1.7 ± 0.6 (0.21 ± 0.13) 5.0 ± 0.8 (0.79 ± 0.13)	1.8 ± 0.2 (0.3 ± 0.07) 5.4 ± 0.9 (0.69 ± 0.07)
'660'	5.4 ± 0.4	5.6 ± 0.6
'610'	48 ± 2.1	46.0 ± 3.4

The relaxation time constant is defined by $A(t) = A_i e^{-t/\tau_i}$, where the absorbance change is the sum of exponential terms. The relative amplitudes are given in brackets near the corresponding relaxation time constants. The experiments were done at room temperature ($22 \pm 1^\circ \text{C}$).

*Suspended purple membrane in water at pH 7.2.

†Thin purple membrane preparation equilibrated with 94% relative humidity.

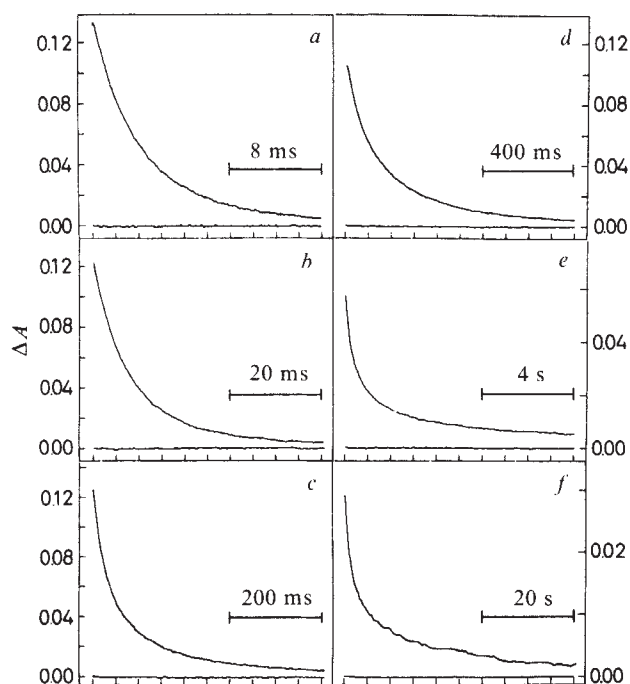


Fig. 1 Oscilloscope traces obtained in flash photolytic measurements of thin purple membrane layers, kept in various hydration states. The traces represent the decay kinetics of '412' nm intermediate formed by a linearly polarised light pulse from a rhodamine 6G laser at 585 nm of 1 μs duration. The samples were excited at right angles to the analysing light, where the plane of the thin purple membrane layers formed an angle of 45° both with the exciting and the analysis light axes. The amplified output of the photomultiplier passed through a variable RC filter to a digital scope, where the filter setting was such as to provide filter relaxation time equal to 0.05% of the oscilloscope's total time sweep width. The transient changes of '412' were recorded directly in absorbance units by the use of a lin-log converter. The flash experiments were performed with thin purple membrane equilibrated with the following relative humidities: a, 94%; b, 90%; c, 83%; d, 75%; e, 43%; f, 7%.

NaCl), or when suspending purple membrane in water-glycerol mixtures. In addition it has been shown that the formation of the '660' intermediate is temperature dependent¹⁸. Thus, the formation of this intermediate can serve as an internal probe of bacteriorhodopsin to environmental conditions, such as temperature, pH or relative humidity. The hydration effects were found to be fully reversible and each

sample was recycled through the various hydration states several times without noticeable irreversible changes.

The results show that the amount of adsorbed water on the purple membrane determines the relaxation times of the '412' decay. The changes in hydration cause a change of the relaxation times over four orders of magnitude (Table 2). It should be added that the rate of the photoactivated formation of the intermediates in the pathway leading to '412' is only changed to a minor degree as a function of the state of hydration. The observed three relaxation times suggest that bacteriorhodopsin undergoes either a three-step conformational change along with the '412' $\rightarrow$ BR⁵⁷⁰ transition or that '412' populates three conformations which decay to that of BR⁵⁷⁰. A similar interpretation was suggested earlier for the biphasic decay of '412' (refs 17, 19).

These effects could in principle be accounted for by a rate-limited diffusion of protons due to the amount and structure of the conducting water layers on the purple membrane or by a reversible conformation change induced by the hydration

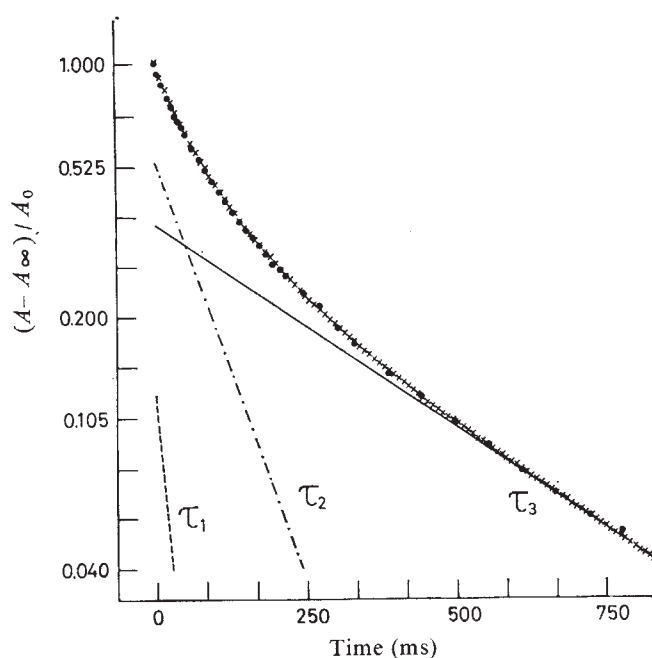


Fig. 2 Evaluation of data from Fig. 1d. The transient absorption $A(t)$ is expressed as a sum of exponentials by $A(t) = A_i e^{-t/\tau_i}$, where A_i is the amplitude of the τ_i relaxation time constant. Closed circles represent the original data points and crosses represent the calculated data points using τ_1 , τ_2 and τ_3 as the relaxation time constants.

Table 2 Relaxation time constants and amplitudes of the decay kinetics of '412' intermediate in thin layer preparations of purple membrane equilibrated in various relative air humidities

Relative air humidity (%)	τ_1	τ_2	τ_3
90	3.5 ± 0.4 ms; (0.63 ± 0.05)*	13.1 ± 1.1 ms; (0.37 ± 0.03)*	—
83	10.2 ± 2.4 ms; (0.18 ± 0.03)	45 ± 3.3 ms; (0.52 ± 0.02)	252 ± 26 ms; (0.31 ± 0.05)*
75	30.5 ± 7.0 ms; (0.11 ± 0.04)	124 ± 33 ms; (0.56 ± 0.08)	503 ± 111 ms; (0.33 ± 0.08)
43	244 ± 100 ms; (0.30 ± 0.06)	1.25 ± 0.4 s; (0.42 ± 0.04)	10.1 ± 3.0 s; (0.28 ± 0.06)
10	0.9 ± 0.2 s; (0.28 ± 0.11)	4.0 ± 1.8 s; (0.35 ± 0.06)	28 ± 8 s; (0.36 ± 0.09)
0	2.3 ± 0.7 s; (0.28 ± 0.03)	15.7 ± 4.5 s; (0.32 ± 0.03)	154 ± 21 s; (0.40 ± 0.4)

*Amplitudes are shown in parentheses.

degree of the purple membrane. The possibility of a diffusional rate-limited proton transfer could be disregarded, however, as it would have imposed a continuous spectrum of relaxation times which is inconsistent with the observed data. The possibility of extreme pH changes due to changes in the amount of adsorbed water can be ruled out, since no spectral shifts of the absorption spectrum of the thin purple membrane layers were observed at different hydrations where a blue shift of the spectrum is expected at high pH and a red shift at low pH (ref. 20 and our unpublished data). A contribution of surface electrical potential changes of the purple membrane suspended in water was analysed by using high ion concentrations (1–4M NaCl) and increasing the pH up to 9. In this extreme case the decay of the '412' was only slowed down by a factor of 50.

Although changes in hydration were shown to control the conformation of proteins²¹, X-ray diffraction studies⁴ of wet and dry specimen of the purple membrane have not shown any significant differences, possibly due to the fact that local changes might escape the sensitivity of the method applied with a 7-Å resolution. Indeed, hydration-induced conformational changes might be expected to occur in much smaller dimensions. Hydration can act either on the immediate environment of the retinal or through inducing a conformational change in the protein which is then transmitted to the retinal-protein interaction region. A possible induced conformational change may also involve an increased formation of internal hydrogen bonding on dehydration, competing for proton transfer, or a conformational induced pK shift of the terminal amino acids. It should be noted that the dehydration process may bring about a large change in the dielectric constant in the vicinity of the proton acceptor. Such a change can shift the pK by two to four units²² and thus change the kinetics by orders of magnitude. Variation of the hydration state of the purple membrane may change the microviscosity of the lipids or even bring to phase separation. The influence of the lipid-protein interaction on the photocycle must be of a secondary importance, however, because no significant difference was found in the kinetics and in proton uptake of proteoliposomes prepared from lipid-depleted protein and from native purple membrane²³. The conformation change is further supported by the observation of a decoupling of the *cis-trans* isomerisation process as a function of hydration²⁴, as well as by the photochemical bleaching of the '412' intermediate even at the lowest humidity state. In addition, dehydration of vertebrate rhodopsin was found to stop the meta I → meta II transition²⁵. Thus in both bacteriorhodopsin and in rhodopsin those conformational changes which involve both protonation-deprotonation reactions and large enthalpy change are those which are most effected by dehydration.

These results stress the importance of the state of hydration in bacteriorhodopsin, presumably as a conformation-controlling event, which might be of general significance for energy transducing membranes especially in the case where deprotonation and protonation processes, coupled to conformational changes are an inherent part of the energy transformation process. The results also indicate that the photochemical reaction is independent from the state of the bulk

solution and is only influenced by the microenvironment of the reaction system which operates in a state of quasi isolation.

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Detection of carcinogen-DNA adducts by radioimmunoassay

COVALENT binding of carcinogen to nucleic acids is believed to be an essential component of the carcinogenic process¹, so it is desirable to have highly sensitive and specific methods for detecting such adducts in cells and tissues exposed to known and suspected carcinogens. We describe here a radioimmunoassay (RIA) capable of detecting nanogram amounts of DNA adducts resulting from the covalent binding of the carcinogen *N*-2-acetylaminofluorene (AAF). AAF and its activated derivative *N*-acetoxy-AAF (*N*-Ac-AAF) are potent carcinogens² and mutagens^{3,4}, and transform cells in culture^{5,6}. DNA obtained from rat liver following *in vivo* exposure to AAF, and DNA exposed *in vitro* to *N*-Ac-AAF contain as the major (80%) adduct *N*-(deoxyguanosin-8-yl)-acetylaminofluorene (dG-8-AAF)^{7–9} and a minor (20%) adduct 3-(deoxyguanosin-*N*²-yl)-acetylaminofluorene (dG-*N*²-AAF)¹⁰. These two types of modification produce markedly different conformational effects on the DNA helix^{11,12}. The major adduct, recognised by

single strand-specific nucleases *in vitro*^{13,14} and DNA repair enzymes *in vivo*^{9,15} was used as the immunogen in this study.

Guanosine (Sigma) and *N*-Ac-AAF (Midwest Research Institute), each at 4.6 mM in 30% ethanol, were incubated for 24 h at 37 °C. The resultant *N*-(guanosin-8-yl)-acetylaminofluorene (G-8-AAF) was separated from the reaction mixture using a 20–100% methanol gradient on Sephadex LH-20 (Pharmacia) and rechromatographed until it gave a characteristic absorption spectrum⁸. The purified product (9 mg) was coupled to bovine serum albumin (BSA, 35 mg, Sigma) by NaIO₄ oxidation followed by NaBH₄ reduction¹⁶, yielding 3.5 mg of covalently bound G-8-AAF ($\Sigma_{305} = 1.5 \times 10^4$). Three rabbits were immunised with G-8-AAF-BSA initially in complete and then in incomplete Freund's adjuvant at weeks 0, 1, 3, 12, 15, 17 and 23. Injections of 0.3 mg hapten were given intramuscularly in the hind legs. Blood was taken from the ear veins before immunisation, at 1 and 2 months, and weekly between 2 and 5 months. All three rabbits produced high levels of antibody by 4 months when assayed by RIA.

For RIA, the tracer, ³H-dG-8-AAF (6 Ci mmol⁻¹) was synthesised from *N*-Ac-AAF and deoxyguanosine (8,5'-³H, 29 Ci mmol⁻¹, NEN), as described above for G-8-AAF. During chromatography on Sephadex LH-20, an initial small radioactive peak, (the presumed dG-N₂-AAF^{9,10}), was discarded and the major radioactive peak (dG-8-AAF) was collected and rechromatographed twice on Sephadex LH-20. A standard curve for RIA was constructed by competing the ³H-dG-8-AAF with unlabelled G-8-AAF or dG-8-AAF, both of which competed equally well. High-titre serum was diluted at least 1:300 (Fig. 1). For equilibrium conditions, ³H-dG-8-AAF, antiserum and inhibitor were incubated at 37 °C for 2 h, followed by goat anti-rabbit-IgG (GAR-IgG, Miles) at 22 °C or 4 °C for a minimum of 1 h. After centrifugation the supernatant was discarded and the pellets were dissolved in 0.1 M NaOH and counted. In these conditions 5–6 ng of unlabelled standard gave a 50% inhibition of antibody-bound label. The standard curves were analysed by computer program, RIAPROG¹⁷, which confirmed the linearity of the RIA by Scatchard plot. An alternate method

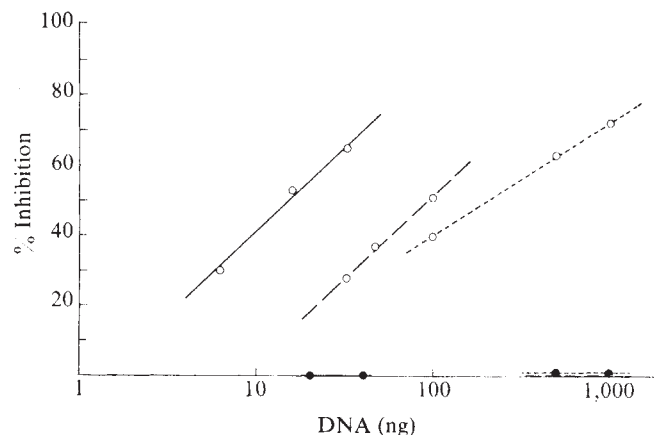


Fig. 2 RIA of *N*-Ac-AAF-modified and non-modified *M. luteus* DNA in differing physical circumstances. *M. luteus* DNA (32 mg, Miles) was heated to 100 °C for 10 min, cooled rapidly in ice and incubated with 36 mg of *N*-Ac-AAF in 17% ethanol in SSC (total volume 42 ml) for 18 h at 37 °C. The reaction mixture was extracted seven times with either, reprecipitated with 70% ethanol to a stable A_{305}/A_{270} , and collected by centrifugation (150,000g, 4 °C, 48 h). Conditions for the RIA, using G-8-AAF as standard, were as in the legend to Fig. 1. Modified (○) or unmodified (●) DNA were assayed without further treatment (---), after heating to 100 °C for 10 min (—) or after enzymatic digestion (—). The enzymatic digestion was performed as follows: samples containing 500 µg DNA were incubated per 0.5 ml of reaction mixture, with 20 units DNase I (Worthington), 5 µmol Mg₂SO₄ and 30 µmol sodium acetate pH 5.5, at 37 °C for 4 h. The pH was then adjusted to 8.5 with (NH₄)₂CO₃ and 20 units venom phosphodiesterase (VP, Worthington) and 1.5 units alkaline phosphatase (AP, BAPC, Worthington) were added to 1 ml of reaction mixture followed by incubation for 18 h at 37 °C. After boiling for 10 min to denature the enzyme protein, samples were cooled on ice, centrifuged (5,000g, 4 °C) to remove the protein precipitate and the supernatant diluted in 0.01 M Tris pH 7.6 for RIA. The actual ng of dG-8-AAF per ng DNA were determined by comparing the values for % inhibition with a simultaneously-run standard curve similar to Fig. 1. The amount of DNA was calculated from A_{260} , by diphenylamine²⁶ reaction and by a fluorescence assay²⁷, all of which agreed within 10%.

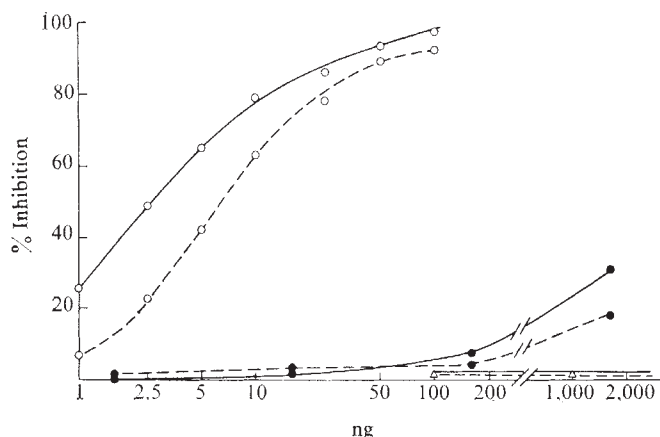


Fig. 1 Radioimmunoassay standard curve at equilibrium conditions (see text) using diluted antiserum (1:1000 in 0.01 M Tris pH 7.6) (dashed line) or purified antibody diluted 1:5 in 0.01 M Tris pH 7.6 (solid line). The reaction mixture containing 0.1 ml antibody, 0.1 ml ³H-dG-8-AAF (6 Ci mmol⁻¹, 40,000 c.p.m.) and various concentrations, in 0.1 ml of G-8-AAF (○), *N*-Ac-AAF (●) or dG (△) was incubated for 2 h at 37 °C. Goat anti-rabbit-IgG (GAR-IgG, Miles) was diluted 1:2 in water and 0.1 ml added per tube. After incubation for an additional hour at 22 °C or 4 °C, the precipitate was collected by centrifugation, dissolved in 0.2 ml 0.1 M NaOH, and counted in Aquasol-2 (NEN), in a Beckman LS-250 scintillation counter. Two to four replicates were assayed per point and values agreed within ± 5%. A blank of about 800 c.p.m., obtained with non-immune serum, was subtracted from each assay. Concentrations of diluted antiserum or purified antibody were chosen such that about 10,000 c.p.m. were bound in the absence of an inhibitor, and this value was taken as 100%.

for increasing the sensitivity of the assay is the use of non-equilibrium conditions, in which the labelled hapten is added for much shorter periods of time, thus enhancing the competition of the test compound. Antibody and unlabelled standard or inhibitor were incubated for 2 h at 37 °C and cooled to 4 °C for 10 min. Tracer was then added, followed 5 min later by GAR-IgG and samples kept at 4 °C for 45 min before centrifugation. In these conditions 1 ng of either G-8-AAF or dG-8-AAF gave a 50% inhibition.

Using the RIA at equilibrium conditions the specificity of the antiserum was tested against deoxyribonucleosides, AAF and related compounds. As shown in Fig. 1, whereas 2–5 ng of G-8-AAF produced a 50% inhibition, up to 100 µg of dG were inactive. *N*-Ac-AAF as well as AAF and *N*-OH-AAF (data not shown) produced a slight inhibition only at a very high concentration, and up to 100 µg of dT, dA, and dC were totally ineffective (data not shown).

Antibody specific to G-8-AAF was purified from the above antiserum by affinity chromatography. Agarose-*adipic hydrazide* (Miles) was coupled covalently to G-8-AAF by NaIO₄ oxidation¹⁸, yielding 3 µmol G-8-AAF per ml packed Agarose. Antiserum was passed through the column and 90% of the antibody activity remained bound. Elution was accomplished either by alternate washes with 0.2 M glycine pH 2.5, and 0.01 M Tris pH 7.6, or with a 5–7 M urea gradient, increased stepwise. The purified antibody showed greater sensitivity for G-8-AAF (2.5 ng gave 50% inhibition at equilibrium) than the unfractionated antiserum, and the same specificity as the diluted antiserum with respect to nucleosides and AAF derivatives (Fig. 1). The amount of purified antibody recovered was

equivalent to 20% of the binding capacity of the starting antiserum.

To define further the specificity and sensitivity of the RIA, the G-C rich DNA from *Micrococcus luteus* was extensively modified by reaction with *N*-Ac-AAF *in vitro* as described in Fig. 2. The amount of dG-8-AAF, when determined by A_{305} , indicated that 28% of the nucleotides contained covalently-bound AAF residues. A similar high degree of modification, representing 70–80% of G substitution, has been previously reported for *M. luteus* DNA reacted with *N*-Ac-AAF¹⁹. But, when assayed initially by RIA this DNA gave values indicating a 1.6% modification (Fig. 2). If the DNA was denatured by heating before RIA, values indicated a 5.5% modification. Finally, when the DNA was hydrolysed enzymatically and the digest assayed by RIA, a 27% modification, corresponding closely to that predicted by the spectral method, was observed (Fig. 2). Thus the antibody recognises only a fraction of the dG-8-AAF residues in intact DNA but quantitative detection is obtained when the DNA is hydrolysed. The 1.6% modification observed before heat-denaturation or hydrolysis might be due to partially denatured regions or AAF binding near the ends of DNA strands. These results are consistent with evidence that most of the covalently-bound AAF residues lie inside the polynucleotide structure stacked with adjacent bases¹¹ and, therefore, presumably shielded from optimum interaction with the antibody. It is of interest that antibodies to other naturally occurring modified nucleosides recognise residues in denatured, rather than native, DNA^{20–23}.

This RIA has also been useful in detecting the extremely low levels of carcinogen modification of DNA that occur in *in vitro* conditions. As shown in Table 1, when cultured primary mouse epidermal cells were exposed to 10^{-4} – 10^{-6} M *N*-Ac-AAF, a concentration-dependent increase in binding of AAF to cellular DNA was detected by the RIA. The results shown were reproduced in four separate experiments, although the extent of binding varied by a factor of 2 with different batches of primary cultures. Factors contributing to this type of variability with primary epidermal cell cultures have been previously discussed²⁴. At an exposure concentration of 10^{-6} M the amount of dG-8-AAF detected in three experiments (data not shown) ranged from 3.5 to 10 μ mol dG-8-AAF per mol DNA-P. These values are similar to those reported for binding measured by the specific

activity of DNA following the exposure of mouse 3T3 cell cultures to 10^{-6} M 14 C-*N*-Ac-AAF for 1 h (ref. 25).

These results indicate that the covalent binding of a chemical carcinogen, AAF, with nucleic acids can be readily detected by RIA with a specific antiserum to the carcinogen–nucleoside adduct. The complete cross-reactivity between G-8-AAF and dG-8-AAF was to be expected since the bovine serum albumin carrier was linked through the 2' and 3' OH groups of the ribose. It is remarkable that our antiserum specifically recognises the AAF–nucleoside complex, but not free AAF, or nucleoside. This specificity is of particular importance for a carcinogen like AAF where two guanosine adducts have been described. Our antigen contained only the C-8 adduct, so it is likely that our antibody detects mainly the corresponding adduct in DNA. Studies are in progress to determine whether or not there is significant cross-reactivity with dG-*N*²-AAF. It is possible that each type of adduct will have immunological specificity and can be independently assayed with high sensitivity during the process of AAF carcinogenesis. The usefulness of this immunological approach may be extended to other types of studies and other classes of carcinogens. Experiments to measure removal of carcinogen by DNA repair processes are in progress, and assays for the possible covalent binding of suspected environmental carcinogens to human tissues should also be feasible.

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Table 1 Detection of dG-8-AAF by RIA in DNA from cells exposed to *N*-Ac-AAF

Concentration of <i>N</i> -Ac-AAF	Content of dG-8-AAF		Exp 2	
	ng per 100 μ g DNA	μ mol per mol DNA-P	ng per 100 μ g DNA	μ mol per mol DNA-P
1.0×10^{-6} M	0.57	3.5	NT	—
1.5×10^{-5} M	2.56 ± 0.6	15.6 ± 3.5	1.6	9.7
5.0×10^{-5} M	5.44	33.1	2.44 ± 0.2	14.8 ± 0.8
5.0×10^{-4} M	13.40	81.5	6.28	38.2

Primary BALB/c epidermal cells were prepared from newborn mice^{24,28}, and 2.4×10^6 cells were plated in roller bottles (Bellco, 825 cm²). On day 3, the indicated concentration of *N*-Ac-AAF dissolved in dimethylsulphoxide (DMSO) or DMSO alone, was added to the medium for 1 h at 37 °C, after which the cells were collected and DNA isolated and purified as previously described¹⁹. The DNA was then hydrolysed enzymatically as described in Fig. 2 and duplicate aliquots containing approximately 25 μ g of DNA and 50 μ g of DNA (0.025 and 0.05 ml respectively) were assayed by RIA using non-equilibrium conditions (see text). Identical results were obtained at both DNA concentrations. A standard curve was constructed from equivalent amounts of untreated and hydrolysed BALB/c epidermal DNA to which were added known nanogram quantities of dG-8-AAF. Control DNA from cultures not exposed to *N*-Ac-AAF did not inhibit the RIA. Values listed in the table refer to DNA from one bottle of treated cells except in cases (*) where the mean $\pm$ the range for three bottles has been given. NT, not tested.

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Specific poly-A-binding protein of 76,000 molecular weight in polyribosomes is not present on poly A of free cytoplasmic mRNP

CYTOPLASM of animal cells contains mRNA associated with ribosomes (in polysomes) and mRNA free of ribosome attachment. Both classes of mRNA occur as ribonucleo-protein (mRNP) complexes¹⁻³. The role of the untranslated pool of free mRNA (referred to as informosomes³) is uncertain. They represent the form in which mRNA exists in transit from the nucleus to the cytoplasm awaiting association with ribosomes³, and in addition the pool of free cytoplasmic mRNA contains inactive (or inactivated) and storage forms of mRNA⁴. It has been established that most of the polysomal mRNP complexes contain predominantly two proteins with approximate molecular weights (MW) of 49,000–52,000 and 73,000–78,000 respectively⁵⁻⁹. It is generally accepted that the larger of these proteins is attached to the 3' poly A tail of

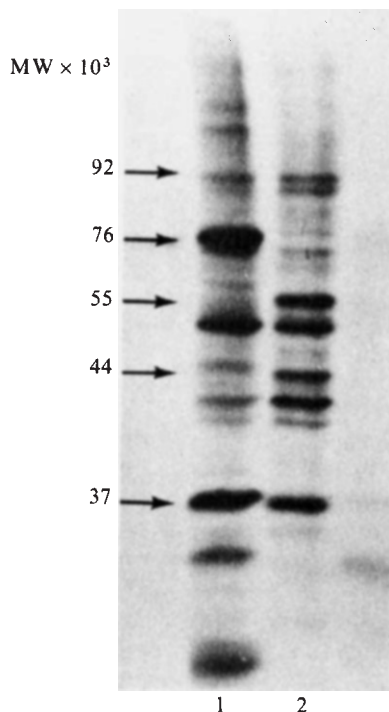


Fig. 1 Autoradiograph of a polyacrylamide gel showing patterns of proteins present in oligo dT-cellulose-purified polyribosomal and informosomal mRNP from Ehrlich ascites tumour cells. Ehrlich ascites tumour cells were labelled with ³⁵S-methionine in the presence of low concentrations of actinomycin D and ethidium bromide (0.04 $\mu\text{g ml}^{-1}$ and 1 $\mu\text{g ml}^{-1}$, respectively) as described previously¹³. Polyribosomes were pelleted through a 2 M sucrose layer containing 0.5 M KCl and free cytoplasmic mRNP was pelleted from the post-ribosomal supernatant in the presence of 0.5 M KCl by a 24-h centrifugation at 200,000g. The polyribosomes and informosomal mRNP pellet were suspended in a 200 mM NaCl, 50 mM Tris-HCl, pH 7.8, 10 mM EDTA solution containing 0.2% Nonidet-P40 and applied onto an oligo dT-cellulose column. Elution of the adsorbed mRNP particles was as described by Lindberg and Sundquist⁷. The formamide eluted mRNP particles were analysed by electrophoresis in sodium dodecylsulphate (SDS) on 7–18% polyacrylamide gradient gels¹⁵. Channel 1, ³⁵S-labelled proteins present in polyribosomal mRNP. Channel 2, ³⁵S-labelled proteins present in informosomal mRNP. The indicated MWs were extrapolated from the positions of marker proteins (not shown): phosphorylase b (MW 92,000), bovine serum albumin (68,000), ovalbumin (45,000), α A₂-crystallin (20,000) and cytochrome c (12,000).

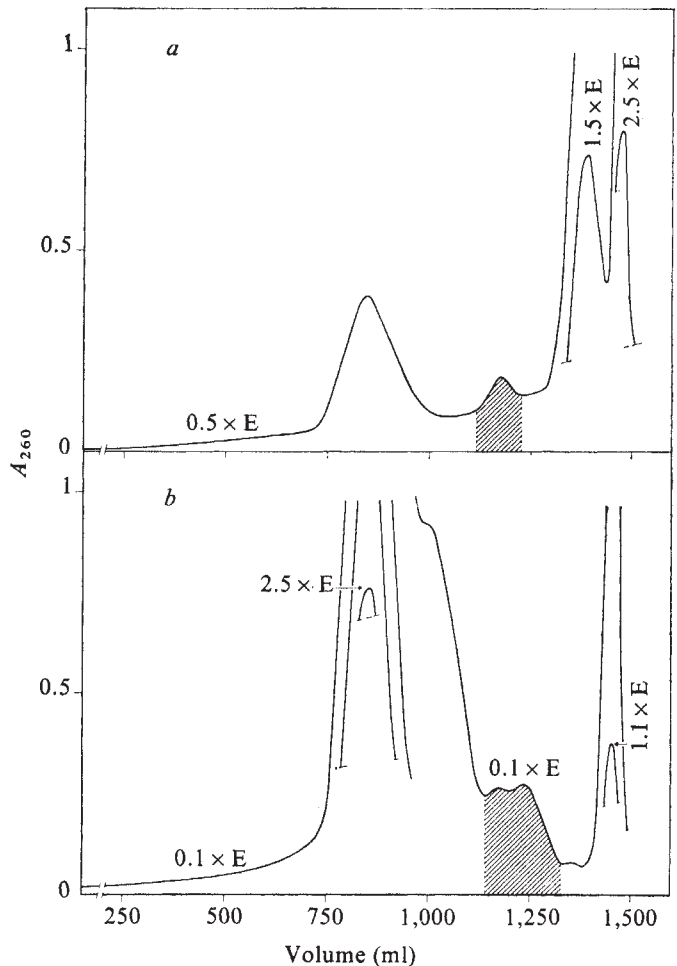


Fig. 2 Zonal centrifugation profiles of EDTA-dissociated polyribosomes (a) and of informosomes (b) from rabbit reticulocytes. Polyribosomes were isolated from rabbit reticulocyte lysates and, after washing with 0.5 M KCl, were suspended in 10 mM Tris-HCl, pH 7.4, containing 30 mM EDTA. The post-polysomal supernatant of the lysate was centrifuged for 24 h at 78,000g. The pellet, containing the informosomal mRNP was suspended in 10 mM Tris-HCl, pH 7.4. About 2,000 A_{260} units of each sample (EDTA-treated polyribosomes and informosomes) were applied onto 0–42% (w/w) zonal sucrose gradients in 10 mM Tris-HCl, pH 7.4. The mixing volume was 750 ml and overlay and sample volumes were 550 and 50 ml, respectively. 180 ml of the gradient was used in the overlay. Centrifugation was carried out at 35,000 r.p.m. for 21 h at 4 °C using the IEC B29 rotor. The gradient was analysed at 260 nm (2-mm flow cell) with a continuous monitoring system and the part of the gradient containing the mRNP particles (hatched area) was pooled.

mRNA¹⁰⁻¹². We present evidence here that the poly A segments of non-polysomal mRNA of Ehrlich ascites tumour cells and of rabbit reticulocytes—in contrast to the polysomal poly A segments¹⁰⁻¹³—do not contain the 76,000 MW protein.

Ehrlich ascites tumour cells were labelled with ³⁵S-methionine in the presence of low concentrations of actinomycin D and ethidium bromide. In these conditions the proteins present in mRNP complexes were labelled whereas little radioactivity was incorporated in the proteins of high salt washed polyribosomes^{13,14}. The oligo dT-cellulose procedure of Lindberg and Sundquist⁷ was used to purify the poly A-containing mRNP present in polyribosomes and informosomes. The 50% formamide eluate was analysed by sodium dodecylsulphate-polyacrylamide gel electrophoresis¹⁵ followed by scintillation autoradiography. The result is shown in Fig. 1. The high degree of similarity between the protein patterns of both classes of mRNP particles is

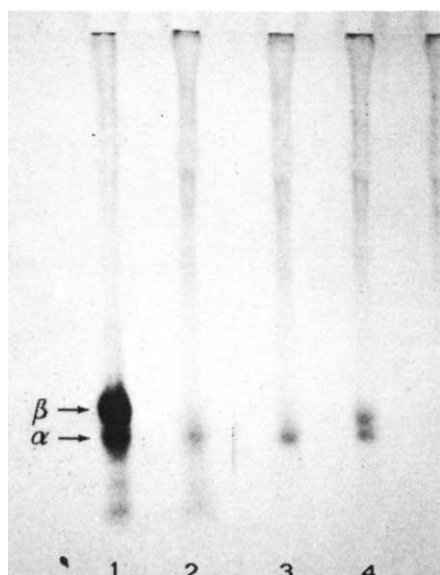


Fig. 3 Electrophoretic analysis of the products of *in vitro* translation of purified informosomal 15S mRNP and 9S polysomal and informosomal mRNA from rabbit reticulocytes. A cell-free system from Ehrlich ascites tumour cells growing in suspension culture¹³ was prepared as described by Hunt²⁵ and treated with micrococcal nuclease as described by Pelham and Jackson²⁶. Purified polysomal and informosomal 9S mRNA and 15S informosomal mRNP were added to the incubation mixture (25 μ l) containing 15 μ l Ehrlich ascites extract and 2 μ l nuclease treated 0.5 M KCl wash of rabbit reticulocyte polyribosomes (initiation factors). Haemin (25 μ M) and other ingredients in the concentration described previously²⁷ were added. Incubation was for 1 h at 30 °C. Part of the incubation mixture was boiled in SDS-sample buffer and loaded onto 13% (w/v) slab gels in a buffersystem described by Weber and Osborn²⁸. Slab gels were prepared for scintillation autoradiography as described before²⁹. Channel 1, products synthesised by polyribosomal 9S mRNA. Channel 2, product synthesised by mRNA isolated from purified informosomal mRNP. Channel 3, product synthesised by purified informosomal mRNP. Channel 4, labelled α - and β -globin markers.

apparent. But, the predominant 76,000 component present in polysomal mRNPs was absent in newly-synthesised informosomal mRNPs. Exhaustive degradation of informosomes from these cells with ribonucleases released poly A-protein complexes containing stretches of poly A of about 90–110 adenyl residues and a predominant 84,000 MW protein¹⁶. (In a Laemmli gel¹⁵ as has been used in this work this protein was visible as a double band around 89,000 MW (Fig. 1, slot 2).) The fact that the poly A segments of these newly-synthesised mRNPs were considerably longer than the polysomal poly A segments in these cells (about 70 adenyl residues¹³) is an indication that we were dealing with young 'transit' mRNPs in contrast to old inactive forms of mRNPs with smaller poly A segments^{17–20}.

To establish whether the old inactive forms of informosomal mRNPs also lack the specific poly A-binding protein of 76,000 MW, polysomal and informosomal mRNP were isolated from rabbit reticulocytes. These cells synthesise predominantly the α and β chains of haemoglobin which are encoded by a 9S mRNA. Another characteristic of these cells is that the free cytoplasmic 9S mRNA primarily codes for the α globin chain^{21,22}. It represents mainly old mRNA characterised by a relatively small poly A segment¹⁷.

Reticulocyte polyribosomes and informosomes were isolated as described in Fig. 2 legend. The polysomal mRNP sedimented as a 15S particle (Fig 2a), while the free cytoplasmic mRNP had S-values ranging from 15 to 20S (Fig. 2b). Both classes of particles contained 9S mRNA that

could be adsorbed on oligo dT-cellulose and eluted with a low salt buffer as described by Aviv and Leder²³. The presence of a poly A tail in the informosomal mRNP was further established by hybridisation with ³H-poly U (ref. 24) (data not shown).

Translation of the polysomal and informosomal mRNP and their respective mRNA was performed in a cell-free system prepared from Ehrlich ascites tumour cells²⁵ treated with micrococcal nuclease as described by Pelham *et al.*²⁶. Figure 3 shows that polysomal 9S mRNA coded for α and β globin, a result that is in agreement with various earlier reports. The major product synthesised by the informosomal mRNP and its 9S mRNA was α globin (slot 3 and 2, respectively). The fact that free cytoplasmic mRNA (mRNP) from rabbit reticulocytes coded primarily for the α globin chain has been described earlier^{21,22} and corroborated the assumption that our 15–20S mRNP preparation was not significantly contaminated by polysome derived mRNA (or mRNP). The observation of Civelli *et al.*³⁰ that duck erythroblast informosomes are not translated (in a wheat germ cell-free system), suggests that unknown components in the cell-free system are required for translation of these particles.

The protein composition of the 15S polysomal and 15–20S informosomal mRNP particles is shown in Fig. 4. Molecular weights were calculated using a series of marker proteins with known MW. In the polysomal mRNP the 76,000 MW protein and two proteins around 50,000 MW were prominent. A series of about six bands between 24,000 and 34,000 MW (four of them more prominent) was also a characteristic feature of the mRNP composition. The latter series of six bands was also present in informosomal mRNP. It is clear, however, that the 76,000 MW protein is not present although the informosomal mRNA contains a 3' poly A tail (see above). The protein bands around 58,000 MW (consisting of at least three proteins as could be seen in more clearly separated electrophoresis patterns) visible in slot D were removed when 15–20S informosomes were washed with 0.5 M KCl. The particles then were converted into predominantly 15S mRNP particles containing α 9S mRNA and the series of six bands which were probably proteins more firmly attached to the mRNA (slot i).

We conclude that poly A-containing informosomal mRNA in Ehrlich ascites tumour cells and in rabbit reticulocytes do not contain the 76,000 MW protein which in these and most other cells is associated with the poly A of polysomal bound mRNA. In view of the fact that the size of the polyadenylate segment of mRNA shortens when the message ages^{17–20} we suggest that both poly A and the protein associated with it are degraded during ageing of mRNA. Newly-synthesised mRNA not associated with ribosomes (for example in Ehrlich ascites tumour cells) has a protein of MW 84,000–89,000 attached to its relatively long poly A segment¹⁶, whereas a 76,000 MW is attached to the poly A of mRNA in the translational phase. Later on, the mRNA (for example the informosomal α -globin 9S mRNA) is released into the pool of untranslated mRNA. In this phase only low-MW proteins, if any, are attached to the shortened poly A segments. It is tempting to speculate that the loss of the 76,000 MW protein and shortening of the poly A tail are cooperative processes which are closely related to the functioning and/or stability of the mRNA.

During the preparation of this manuscript Scherrer's group³¹ published the protein composition of informosomal mRNP from duck erythroblasts. In agreement with our observations they also found no 76,000 MW protein associated with poly A-containing informosomal mRNP.

We thank Drs F. A. Asselbergs and A. J. M. Berns for help and advice and A. P. M. Janssen for technical assistance. This work was supported in part by the Netherlands

MW $\times 10^{-3}$

130 →
92 →
68 →
45 →
20 →
12 →

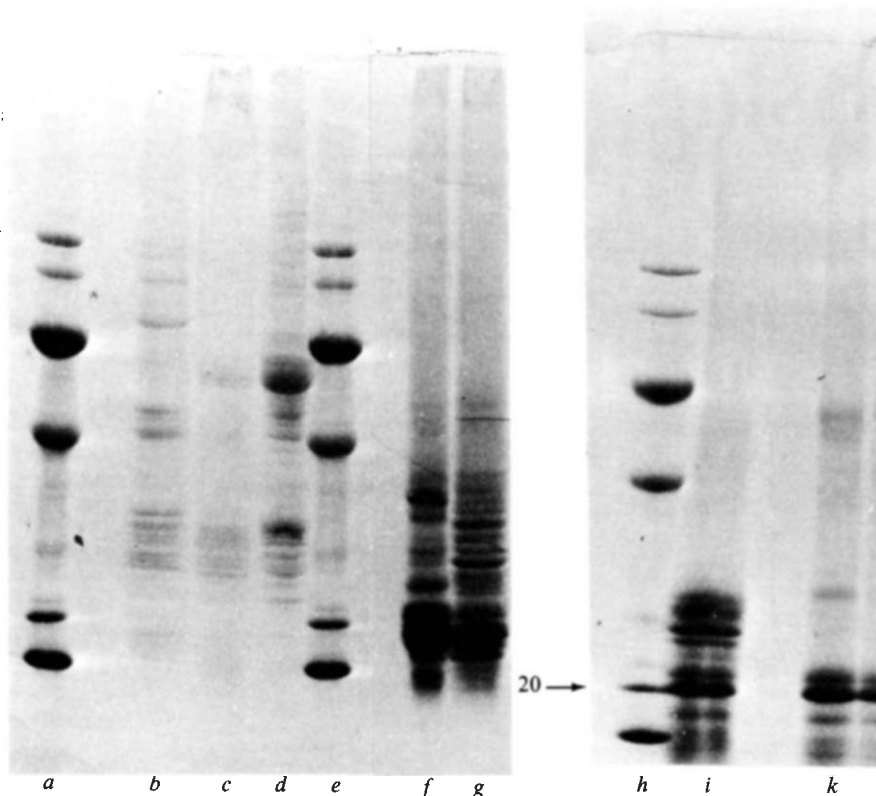


Fig. 4 Analysis of proteins present in purified polysomal and informosomal mRNP particles from rabbit reticulocytes. mRNP particles were dissolved in SDS-sample buffer, boiled and loaded on to a 7–18% polyacrylamide gradient gel containing SDS¹⁵. Channels *a, e, h*, marker proteins: β -galactosidase (MW 130,000), phosphorylase b (92,000), bovine serum albumin (68,000), ovalbumin (45,000), α_2 -crystallin (20,000), cytochrome *c* (12,000). *b*, Polysomal 15S mRNP. *c*, Informosomal mRNP after a 0.5 M KCl wash (see also *i*). *d*, Informosomal mRNP before the 0.5 M KCl wash. *f*, EDTA-treated 40S ribosomal subunits. *g*, EDTA-treated 60S ribosomal subunits. *i*, Informosomal mRNP after a 0.5 M KCl wash (double band at 20,000 is carrier α -crystallin). *k*, 0.5 M KCl wash of informosomal mRNP (double band at 20,000 carrier is α -crystallin).

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Corrigendum

In 'Voltage signal of photoreceptors at visual threshold' by G. L. Fain, A. M. Granda and J. H. Maxwell (*Nature* **265**, 181; 1977), the ordinate label of Fig. 1 should read: $\log \Delta I_T$ ($\mu\text{J cm}^{-2} \text{ flash}^{-1}$). Likewise, the abscissa label of Fig. 1 should read: $\log \Delta I_T$ ($\mu\text{J cm}^{-2} \text{ s}^{-1}$). This is simply a labelling error and has no effect on any numbers in the text. The authors are grateful to Dr G. S. Wasserman of Purdue University for pointing out this error.

Errata

In the article 'Corrected age of the Pliocene/Pleistocene boundary' by B. U. Haq, W. A. Berggren & J. A. Van Couvering, *Nature* **269**, p. 483, the legend to Fig. 5 should read . . . Pliocene/Pleistocene calcareous plankton biochronology in deep-sea cores and estimated chronostratigraphic position of Calabrian sequences. The extinction of *Discoaster brouweri* occurs at about 1.8 Myr (*) in one of the cores studied (V12–18). The upper limit of this species, as shown here, may thus be somewhat younger than the actual extinction datum, due to reworking at the depositional interface. ○, Atlantic only.

In the letter 'Corollary discharge to cockroach giant interneurons' by F. Delcomyn, *Nature* **269**, p. 160, line 17 in paragraph 4 should read . . . When a strong stream of air is suddenly turned on a quiet . . .

In the letter 'Direct measurements of secondary currents in river bends' by J. C. Bathurst, C. R. Thorne and R. D. Hey, *Nature* **269**, p. 504, line 2 in paragraph 6 should read . . . angle φ to the longstream axis. φ defines the vector of the . . .

matters arising

How did barium titanate particulates stick together in the Nebula?

MASUDA and Tanaka¹ in their recent letter have raised this question. Seeking explanation in the ferroelectric (FE) properties of BaTiO₃ we should ask now more specifically whether FE particles, similar to magnetic particles², may provide preferred nuclei for accretionary processes.

A FE particle acquires charge densities ($\sim 10^{-4}$ C cm⁻²) much greater than is found with ordinary electrostatic charging, for example, that arising from the termination of crystal structure in unsaturated and dangling bonds which become rapidly screened by adsorption. To produce the kind of homogeneous polarisation which FE particles develop at their Curie temperature T_C (393 K for BaTiO₃) in an ordinary dielectric would require fields of 10^6 – 10^8 V cm⁻¹. The distorted surface layers observed on sub- μ m FE particles³ indicate that the particles do not find it easy to screen their surface charges by adsorption or internal electrical conduction. Moreover, T_C of particles $< 1 \mu$ m may exceed 700 K. We may thus expect the potential corresponding to a FE particle to be much greater than the 1–30 V considered feasible for normal electrostatically charged interplanetary grains^{4,5}.

The potential energy of a small uniformly polarised BaTiO₃ crystal, represented by a doublet p_i , outside a complex formed by a collection of many uniformly polarised crystals and additional charge distributions corresponding to non-FE constituents, is determined by the normal component p_n at the surface of the complex. To determine the capture cross section⁶ we consider a complex of radius r and surface polarisation p_n moving with velocity u . A doublet p_i with mass m_i and velocity v makes its closest approach at distance R from the centre of the complex and is captured in a grazing orbit. On the supposition of conservation of energy and momentum we obtain for the ratio $K = (\pi R^2)/(\pi r^2)$

$$K \approx 1 + \frac{p_i p_n (1 + 3 \cos^2 \theta)^{1/2}}{4 \pi r^3 m_i v^2}$$

Taking p_i and p_n to be of the order of $\approx 5 \times 10^{-5}$ C and $v \approx 1.5$ cm s⁻¹ gives $K \approx 5 \times 10^7$ for a 0.15μ m particle.

The micrographs, presented by Masuda and Tanaka¹, show a large number of small composite particulates with a narrow size distribution around 0.15μ m and two conspicuous clusters of $\sim 5 \mu$ m. If we were to interpret the latter as records of enhanced

capture cross sections of BaTiO₃ in the early environment within the nebula, then these would suggest enhancement factors between 1.7 and 10^3 and $\sim 10^7$ depending on the size of the initial condensates.

While an enhanced capture cross section may explain why FE particles will stick together when available, it does not explain the high Ba abundances found in eucrites and in the Allende meteorite^{7,8} as such. In view of the wide gap between the condensation temperature of $\sim 1,680$ K and T_C those high BaTiO₃ concentrations which lie well above statistical fluctuations suggest selective spatial separation and enrichment. On account of the very high dielectric constant, even above T_C , the radiation pressure on BaTiO₃ condensates must have been greater than for most other particles of similar density and cross section. (Reflectivity coefficient for carbonaceous chondrites is ~ 0.04 – 0.07 , (ref. 8) for BaTiO₃ ~ 1 .) For particles of $\sim 0.1 \mu$ m the force due to radiation pressure outweighs by far the gravitational attraction. Assuming the latter to be balanced by the centrifugal force in the orbital motion, the spatial distribution of condensates will be determined primarily by the radiation pressure and the Lorentz forces due to a solar or interplanetary magnetic field^{10,11}.

Considering conditions at 4 a.u. and assuming a moderate particle potential of 100 V in a uniform magnetic field of 5×10^{-5} gauss we obtain, in Table 1, for the forces due to radiation (f_R), gravitation (f_G) and Lorentz forces (f_L) the following values for particles of 0.125 , 1 , and 5μ m.

Table 1 Forces acting on FE particles

0.125μ m (μ N)	1μ m (μ N)	5μ m (μ N)
f_R 2.6×10^{-14}	1.8×10^{-12}	4.5×10^{-11}
f_G 1.7×10^{-14}	1.1×10^{-11}	1.4×10^{-9}
f_L 8.5×10^{-13}	5.4×10^{-12}	1.3×10^{-11}

We may thus infer that FE particles up to 1μ m may settle out preferentially towards the inner part of the solar nebula. As particles aggregate into clusters of 5μ m, however, the radiation pressure wins out and depending on growth and ablation, larger particles are thus likely to equilibrate at 4 a.u. or to be swept out to greater distances.

At distances ≥ 1.75 a.u. with grain temperatures < 300 K a BaTiO₃ grain should be in its orthorhombic or rhombohedral modification. It would, therefore, be interesting to determine the crystal structure

of BaTiO₃ grains in the Allende meteorite. Moreover, the small dispersed particles as well as the larger clusters should exhibit FE polarisation and hysteresis^{11–13} and it would thus be instructive to determine their *in-situ* Curie temperature. Structure and transition temperatures may give away further clues as to how BaTiO₃ grains came to be a vastly enriched constituent in the composite matrix forming the Allende meteorite.

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Diversity of deep-sea benthos

THE remarkable diversity of the deep-sea benthos is now well known, and Wolff¹ has contrasted two theories to explain this. One is Sanders² theory of niche specialisation, the other is Dayton and Hessler's³ theory of biological disturbance, including disturbance by predators. One point that may discriminate between theories explaining diversity is that it seems that many deep-sea benthic forms have long life expectations and low reproductive rates¹. I point out here that these life history characteristics are found in another quite distinct group of high diversity, which also lives in a habitat with very little seasonal change—tropical forest birds. Snow⁴ reviews much of what is known of the population dynamics of such birds, particularly the fruit-eating ones. The life history seems to be characterised by small clutch size and yet a very high early mortality, particularly of eggs and nestlings, and a low mortality thereafter. This early mortality is caused by predators. Snow says "Once a bird has survived the early weeks of life, the tropical forest is a

much safer place to live in than the woods of temperate regions. Crises are rare; day after day passes in a peaceful, almost monotonous routine". Is it not possible that a similar pattern of life history mortality, high when very young, low thereafter, and a lack of seasonal changes, have enabled the evolution of high diversity both in the tropical forest and in the deep sea? If this is so, the critical specialisations would presumably be in minimising the early mortality in various ways, and small brood sizes may be a necessary part of these specialisations, by making detection by predators more difficult⁴. There would then be much less pressure for niche specialisation among the adults.

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Contemporaneity of late Cretaceous extinctions

THE dramatic extinctions at the end of the Cretaceous, both on land and in the sea, have long provoked speculation, but there is little direct evidence. Their existence is why the Cainozoic Era is distinguished from the Mesozoic, and in practice they are used in stratigraphy to make the Cretaceous–Palaeocene boundary. If the extinctions occurred at different times in different places, this practice would have to be revised.

Butler *et al.*¹ presented magnetostratigraphic evidence that dinosaur extinction in northern New Mexico occurred slightly later than the marine extinctions in central Italy. They seem to generalise this difference to all marine and terrestrial rock sequences, but this may be incorrect.

On the basis of extensive evidence of diverse kinds from Montana, we have been able to reconstruct ecologically the process of terrestrial extinctions there (refs 2–4 and unpublished). The time scale was ecologically very long but geologically very short (10^4 – 10^5 yr), just as in marine sequences^{5,6}. The process, which affected marsupials almost as severely as dinosaurs but left the freshwater community entirely unaffected, was a protracted ecological succession which seems to have operated mainly by diffuse competition with placental and multituberculate mammals. This invading mammalian community^{7,8} soon expanded worldwide to give rise to most of our familiar Cainozoic mammals.

We have predicted^{2,3} that terrestrial extinctions farther south occurred later than those in Montana, but we were able to find² only two flimsy data relevant to

this prediction. Both were from South America and both supported the prediction. The extinctions in Montana were probably not the earliest, as dinosaurs occurred at least to about 75°N palaeolatitude^{2,9} and we expect that dinosaurs disappeared here a little before they did near 50°N in Montana.

It would be a remarkable coincidence if the great marine and terrestrial extinctions did not have causal elements in common. We believe that these extinctions were in part synchronous but that the terrestrial extinctions moved south as the rapidly evolving placental mammals expanded out from their ancestral temperate community. Dinosaurs may well have survived into the Eocene in India², which was an island during the critical interval.

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Do fatty acids inhibit gibberellin-induced amylolysis?

BULLER *et al.*¹ reported that fatty acids inhibit gibberellin-induced amylolysis in barley endosperm. Their experiments as described do not show if the effect is on induction of amylase by gibberellin, on amylase activity, or on resistance of starch to amylase attack, although they discuss results as "the exceptional efficacy of pentanoic (C_5) and nonanoic (C_9) acids in overcoming the effect of GA...".

Mitchell and Zillmann² showed that fatty acids increased flour and starch pasting ability, and some years ago I measured similar effects. In the Amylograph³, with the naturally occurring enzymes inactivated³, a wheat flour had maximum paste viscosity 1,205 Brabender Units (BU). Fatty acid additions (500 mg each to the mixture of 60 g flour and 440 ml water) gave the follow-

ing increases of maximum viscosity, confirming Mitchell and Zillmann's observations: C_6 , 45 BU; C_7 , 85 BU; C_8 , 160 BU; C_9 , 125 BU; C_{10} , 10 BU and C_{11} , 5 BU.

If the flour enzymes (including amylases) were not inactivated, then C_8 fatty acid had remarkably greater effect. Adding 500 mg (58 μ mol per g flour, or 7.9 mM) increased viscosity from 495 BU to 975 BU. This effect for very low amylase activity suggests that fatty acid altered starch properties and made it more resistant to enzymatic breakdown. Commercial glyceryl monostearate (GMS) has a similar effect but GMS plus C_8 acid was no more effective than one component, nor did glyceryl monocaprylate strengthen starch more markedly than equimolar amounts of C_8 acid or GMS.

I suggest that fatty acids about C_8 can make starch more resistant to amylase degradation. Thus the effect demonstrated by Buller *et al.* could be of fatty acid making starch more resistant to amylolysis rather than an effect associated with gibberellin.

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BULLER AND REID REPLY—We have reported that saturated fatty acids of chain length around C_9 are potent inhibitors of gibberellin-triggered amylolysis in embryo-free barley half-grains¹. We are aware that the efficacy of these acids "in overcoming the effect of GA" might be due either to their interfering with some aspect of the production, activation or secretion of amylolytic enzymes or to their inhibition of the amylolytic enzymes themselves. We are now investigating the biochemical mechanism(s) of fatty acid action.

In experiments carefully controlled to eliminate fatty acid effects on amylase activity in our assay systems we have observed that the fatty acids of chain lengths C_7 , C_8 and C_9 at 5×10^{-4} M completely inhibit the release of amylase activity from gibberellin-treated barley aleurone layers. This suggests that the fatty acids can interfere, directly or indirectly, with amylase 'induction' or release.

Yet fatty acids can also inhibit the activity of amylolytic enzymes. Nonanoate (C_9) at 10^{-3} M reduces by about 60% (relative to a citrate control) the

rate of production of reducing equivalents from a solution of potato starch by a crude soluble 'amylase' prepared from germinated barley (N. Harris and J. S. G. R., unpublished).

Our results to date, therefore, suggest that fatty acids of chain length around C_9 inhibit both the production and the activity of amylolytic enzymes. Both effects undoubtedly contributed to our earlier observations¹.

Meredith's suggestion that fatty acids might interact with starch, making it less susceptible to amylolysis, adds a further variable to an already complex problem. Any observed inhibition by fatty acids of amylolytic activity could be attributed either to a fatty acid-enzyme interaction or to a fatty acid-starch interaction as proposed by Meredith. We cannot yet assess the relative importance of the two effects in our assays, and we point out that even the experimental data presented by Meredith can be reinterpreted in terms of a fatty acid-enzyme interaction.

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An imbricate thrust model for Southern Uplands of Scotland

THE timing of the letter by McKerrrow, Leggett and Eales¹ is curious in that the model is not new. It was anticipated in concept by Mitchell² and in detailed demonstration by Fyfe and Weir³. In addition information given within the article includes details of the sequences seen along the British Gas pipeline, and a structural analysis from the Moffat area by Eales. The former is interesting although unexceptionable, apart from the fact that it sets aside without justification previous evidence that not all the Ordovician volcanics are of Arenig age⁴⁻⁶. The structural analysis is unsubstantiated but, more importantly, it ignores structural sequences already established from many other areas in the Southern Uplands and Ireland, which are supported by recent studies^{7,8}.

The history of Cockburnland also requires clarification. Originally proposed⁹ as the largely ophiolitic source for the Ordovician flysch of the Southern Uplands, it has been criticised as inadequate to provide the accumulated volume of sediment⁹. The

name was retained, but was used instead to designate the landmass uplifted in Llandovery times¹⁰ south of the nascent Southern Uplands Fault, and now considered to represent the emergence of an evolving accretionary prism. Obduction of oceanic crust towards or over the Laurentian front¹¹ would create a sedimentary source adequate to provide the known volume of Ordovician flysch, thus dispelling the objection to an Ordovician Cockburnland. We consider that application of the name to the developing accretionary prism can be justified only by assuming geographical continuity with the remnants of the original structure.

Furthermore, the model prescribes progressive sedimentation of coarse clastics from north west to the south east. This must be an over-simplification, in that for at least part of the Llandovery the Moffat shale belt was bounded to north west and south east by thick sandy sequences¹².

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McKERRROW, LEGGETT AND EALES REPLY.—The timing of our contribution was the result of work carried out in 1976 on new exposures in the British Gas pipeline. The model relating the structure of the Southern Uplands to contemporary subduction was first illustrated by Mitchell and McKerrrow¹; this model was based, in part, on earlier work by Craig and Walton², Toghill³, Kelling⁴ and Fyfe and Weir⁵.

Our contribution did not imply that all the Ordovician volcanics in the Southern Uplands are of Arenig age. Those near Abington lie below Arenig cherts, while those at Coulter underlie Llandeilo cherts; the cherts in both areas may well be much younger than the basalts below them. In addition, there are other volcanics interbedded with greywackes (like those at Wrae

and Bail Hill), but these are not oceanic basalts.

The structural analysis (Eales, unpublished) was applied specifically to the incompetent Moffat Shale facies where it occurs at the base of major thrust sheets. It is only in this facies, with its classical graptolite faunal successions, that refined stratigraphy can be used as a basis for structural synthesis. Other local structural sequences (for example, Rust⁶; Weir⁷) are generally consistent with this analysis, but they lack detailed stratigraphic control.

Walton⁸ gave the name Cockburnland to the emergent "chain stretching north-eastwards along the northern margin of the present Southern Uplands" which provide a source for the Llandeilo and Caradoc rocks of the area around Glen App. He also used the same name for the Llandovery landmass which provided sediment both northwards (to the Silurian of the Midland Valley inliers) and southwards (to the Central and Southern Belts of the Southern Uplands). The 'Cockburnland' shown in our contribution (Fig. 3) is the second of these two land areas. It is not clear whether the earliest Ordovician greywackes were derived directly from previously obducted ophiolites lying to the north of the Southern Upland Fault (as Walton and Weir suggest), or whether they were derived from previously accreted ocean floor volcanics and cherts on the inner trench wall.

Our model implies that most of the sediments in the Southern Uplands were deposited on the floor of (or on the margin of) the Iapetus Ocean. Even though coarser sediments occur in the Llandovery and Wenlock beds exposed in the Southern Belt of the Southern Uplands, this does not imply any south-east boundary to the 'Moffat Shale belt', it merely shows that coarser sediments were being deposited on the Iapetus Ocean at this time.

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